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Review

Assessment of TRPM7 functions by drug-like small molecules

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ABSTRACT

Transient receptor potential cation channel subfamily M member 7 (TRPM7) is a plasma membrane ion channel linked to a cytosolic protein kinase domain. Genetic inactivation of this bi-functional protein revealed its crucial role in Ca^{2+} signalling, Mg^{2+} metabolism, immune responses, cell motility, proliferation and differentiation. Malfunctions of TRPM7 are associated with anoxic neuronal death, cardiac fibrosis, tumour progression and macrothrombocytopenia. Recently, several groups have identified small organic compounds acting as inhibitors or activators of the TRPM7 channel. In follow-up studies, the identified TRPM7 modulators were successfully used to uncover new cellular functions of TRPM7 in situ including a crucial role of TRPM7 in Ca^{2+} signaling and Ca^{2+} dependent cellular processes. Hence, TRPM7 has been defined as a promising drug target. Here, we summarize the progress in this quickly developing field.

Contents

1. Physiological and pathophysiological roles of TRPM7

TRPM7 contains a transmembrane channel segment fused to a cytosolic α-type serine/threonine protein kinase domain [1–5]. In analogy to other TRP channels, the channel domain of TRPM7 comprises six transmembrane helices (Fig. 1). A short stretch of amino acids between the 5th and 6th helices contains a predicted pore helix followed by an assumed pore loop (Fig. 1). Pore loops of four TRPM7 channel subunits contribute to a common ion selectivity filter, highly selective for divalent cations. The long cytosolic N-terminus of TRPM7 comprises a set of domains that are conserved among the TRPM gene family, but have no apparent primary sequence homology to other proteins. However, 3D modelling predicted that these domains fold into ankyrin repeats, which are frequently present in TRP channels [6]. The C-terminus of TRPM7 contains a highly conserved transient receptor potential (TRP) domain, a coiled-coil domain, a kinase substrate domain followed by a kinase domain. Among known ion channels and protein kinases, only TRPM7 and its homologous protein TRPM6 are ion channels covalently linked to kinase domains [1,6–11].

The channel segment of TRPM7 forms a constitutively active ion channel highly selective for divalent cations such as Zn^{2+}, Ca^{2+} and Mg^{2+} [1,2,12,13]. The molecular mechanisms governing TRPM7 channel gating are still not completely understood. There are two prevailing models relying on the following observations. First, perfusion of cells with a Mg^{2+}-free internal solution induces TRPM7 currents indicating that cytosolic Mg^{2+} and Mg·ATP are physiological negative regulators of TRPM7 channel activity [1,14,15]. Experiments with truncation mutations deleting C-terminal segments of TRPM7 (Fig. 1) revealed that the kinase and coiled-coil domains modulate the sensitivity of the TRPM7 channel to Mg^{2+} and Mg·ATP [14,16,17]. The second model is predicated on the finding that the TRPM7 channel is regulated by the levels of phosphatidylinositol-4,5-bisphophate (PIP_2) in the plasma membrane [18]. Thus, activation of phospholipase C via

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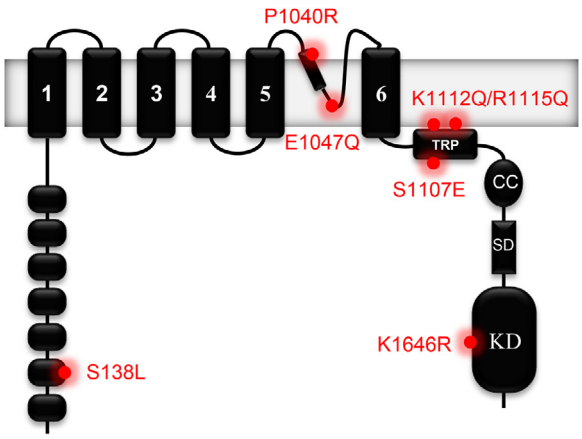

Fig. 1. Domain topology of the murine kinase-coupled channel TRPM7. TRPM7 contains a large cytosolic N-terminus, six transmembrane helices (*1–6*), a conserved transient receptor potential (*TRP*) domain, a coiled-coil (*CC*) domain, a kinase substrate domain (*SD*) and a kinase domain (*KD*). Red dots indicate the location of point mutations that were highly instrumental in probing of TRPM7 functions.

G protein-coupled receptors entails depletion of PIP_2 and, subsequently, inhibition of the TRPM7 channel even in the absence of Mg^{2+} [18]. It was suggested that cytosolic Mg^{2+} can interact with negatively charged PIP_2 leading to suppression of TRPM7 currents [19], implying that both metabolites, PIP_2 and Mg^{2+}, act on TRPM7 through a common mechanism. Xie et al. [20] found that neutralization of basic residues in the TRP domain (Fig. 1) results in a non-functional or dysfunctional version of the TRPM7 channel with a diminished sensitivity to PIP_2. Our laboratory has recently shown that the TRP domain has a remarkable role in the inhibitory effects of Mg^{2+} and Mg·ATP on the TRPM7 channel since a point mutation of a conserved serine residue in the TRP domain (Fig. 1) is sufficient to create a constitutively active TRPM7 channel insensitive to cytosolic Mg^{2+} [21]. Hence, the TRP domain of TRPM7 makes a major contribution to the regulation of channel gating.

Several noteworthy mutant versions of TRPM7 have been generated shedding light on the functional role of distinct TRPM7 domains. Mutations S138L and P1040R in mouse TRPM7 correspond to missense mutations in human TRPM6 associated with an inherited disease known as primary hypomagnesemia type 1, intestinal (HOMG1) or hypomagnesemia with secondary hypocalcemia (HSH) [8,9,22,23]. S138L disrupts the assembly and surface trafficking of TRPM7 channel complexes [10]. P1040R results in a dominant-negative TRPM7 channel subunit [24]. E1047 in TRPM7 is located in the presumed ion selectivity filter of the channel's pore-forming segment. The E1047Q mutation in TRPM7 results in an active channel permeable to monovalent cations and impermeable to divalent ions including Ca^{2+} and Mg^{2+} [25,26], thus, recapitulating the unique characteristics of TRPM4 and TRPM5 channels [27,28]. The TRPM7 S1107E mutation gives rise to a constitutively active channel, insensitive to PIP_2 and intracellular Mg^{2+} [21]. The positively charged residues K1112 and R1115 were reported to be necessary for PIP_2 dependent gating of TRPM7. Accordingly, the K1112Q/R1115Q double mutation in TRPM7 ablates channel activity [20]. K1646 is a highly conserved residue, which forms hydrogen-bonding interactions with the α-phosphate group and the adenine ring of ATP in the catalytic site of the kinase [4]. Consequently, the 'kinase-dead' K1646R mutation fully blocks phosphotransferase activity of TRPM7 kinase [16,29].

TRPM7 is a ubiquitously expressed protein [30–33]. Endogenous TRPM7 currents were detected in all cells investigated so far [30–33]. Genetic disruption of TRPM7 in cultured cells revealed that TRPM7 regulates cellular Mg^{2+} levels [10,16,34–37], cell motility [38–45], proliferation/cell survival [1,16,35,46–48], differentiation [49–51], mechanosensitivity [39,52,53], exocytosis [54], immune responses [43,55] and cytoskeletal architecture [56,57]. In addition, there is mounting evidence to suggest that TRPM7 is involved in anoxic neuronal death [58], hypertension [59,60], neurodegenerative disorders [61,62], atrial fibrillation [63], cardiac fibrosis [63], and tumor growth/progression [64–71]. Genetic association studies in humans revealed that TRPM7 may serve a role in myocardial repolarization [72]. Experiments with *Trpm7* gene deficient mice and zebrafish and genetic association studies in humans showed that TRPM7 is required for early embryonic development [34,73–76], thymopoiesis [73], morphogenesis of the kidney [75], cardiac rhythmicity [77], cardiac repolarization [78], systemic Mg^{2+} homeostasis [34], thrombopoiesis [79] and mast cell degranulation [80].

As mentioned above, the TRPM7 channel is highly permeable to Ca^{2+} and Mg^{2+}, and it has been hypothesized that influx of both cations may be relevant for the physiological role of TRPM7 [1,2,12]. Several independent studies have shown that cells deficient in TRPM7 develop Mg^{2+} deficiency and, consequently, growth arrest [16,34,36,37]. In addition, transient ablation of TRPM7 by RNAi silencing or by pharmacological modulators (see below) revealed that TRPM7-mediated Ca^{2+} influx impacts numerous Ca^{2+} signaling events, for instance, Ca^{2+} oscillations [81,82], store-operated Ca^{2+} entry (SOCE) [83] and Ca^{2+} flicker activity [39].

The functional role of the TRPM7 kinase is not well understood at present. In vitro studies demonstrated that TRPM7 kinase is able to phosphorylate serine/threonine residues of annexin A1 [84], myosin II isoforms [56], eEF2-k [85] and PLCγ2 [86]. Furthermore, multiple residues located in a 'substrate' segment of TRPM7 (Fig. 1) are autophosphorylation targets of the kinase domain [87–89]. Recently, the intriguing observation has been made in immune cells that the TRPM7 kinase moiety can be cleaved from the channel unit by caspases during Fas-receptor stimulation [90]. The truncated TRPM7 channel exhibited increased activity and potentiated Fas-receptor signalling [90]. In another study [13], the cleaved TRPM7 kinase domain was detected in many tissues and cell lines. The authors reported that the portion of TRPM7 encoding the channel domain was eliminated by unknown mechanisms, whereas the released kinase domain translocated into the cell nucleus to phosphorylate histones resulting in changes of the chromatin modification landscape [13]. The physiological relevance of this particular role of the TRPM7 kinase still remains to be delineated, because, in contrast to a global TRPM7 null mutation, mice carrying the 'kinase-dead' point mutation did neither present with aberrant embryonic development nor with any apparent alterations in autonomic physiological or behavioural processes [29]. An independent study showed that mice harbouring the 'kinase-dead' point mutation were more resistant to dietary Mg^{2+} deprivation in terms of overall survival and develop a mild form of organismal Mg^{2+} deficiency [37]. These results were interpreted to mean that TRPM7 kinase might be involved in adaptive systemic responses to Mg^{2+} deficiency [37].

2. Drug-like compounds inhibiting the TRPM7 channel

In light of the wide range of physiological and pathophysiological roles ascribed to TRPM7, there is a pressing need to identify reliable drug-like molecules allowing for the dissection of channel versus kinase activity in situ and under in vivo conditions. Recently, several laboratories identified various small organic compounds acting as blockers of the TRPM7 channel. Fig. 2 shows the structures of most frequently used TRPM7 inhibitors. In the current manuscript, we do not discuss metabolites and exogenous agents behaving as unspecific channel modulators such as spermine [91],