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Gene Section
Review

ORAI3 (ORAI calcium release-activated calcium modulator 3)

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Abstract

Review on ORAI3, with data on DNA/RNA, on the protein encoded and where the gene is implicated.

Identity

Other names: TMEM142C

HGNC (Hugo): ORAI3

Location: 16p11.2

Note

ORAI3 is a member of the ORAI family proteins discovered in 2006 as the essential pore-forming components of the low-conductance, highly Ca^{2+}-selective CRAC channels whose activation is dependent on depletion of the endoplasmic reticulum Ca^{2+} stores (Feske et al., 2006; Vig et al., 2006; Zhang et al., 2006).

In Greek mythology, the ORAI are the keepers of the gates of heaven: Eunomia (Order or Harmony), Dike (Justice) and Eirene (Peace).

DNA/RNA

Description

ORAI3 is encoded by the gene TMEM142C (HUGO Gene Nomenclature Committee).

The ORAI3 gene is located on chromosome 16 in the p11.2.

Transcription

Size of ORAI3 transcript: 2.2 kb; NCBI ORAI3 mRNA model: NM_152288.

All three ORAI isoforms are widely expressed at the mRNA level and can be incorporated into the plasma membrane when ectopically expressed. Broad expression of ORAI3 transcripts has been shown by Northern blot analysis: ORAI3 transcripts are expressed in heart, brain, kidney, thymus, lung, spleen, skeletal muscle, small intestine, as well as in primary aortic endothelial cells and bone marrow derived mast cells (Gwack et al., 2007). ORAI3 appears to be the only family member that is strongly expressed at the RNA level in brain. (ORAI2 transcripts are prominent in kidney, lung, and spleen (Gwack et al., 2007)).

Transcripts expression

In immune cells, transcripts taken from isolated primary $CD3^+/CD4^+$ cells (Th-lymphocytes), $CD3^+/CD8^+$ cells (Tc-lymphocytes), $CD19^+$ cells (B-lymphocytes) and BMMC showed that ORAI3 expression is readily detectable in Th^-, Tc^-, and B^- lymphocytes and BMMC (Gross et al., 2007).

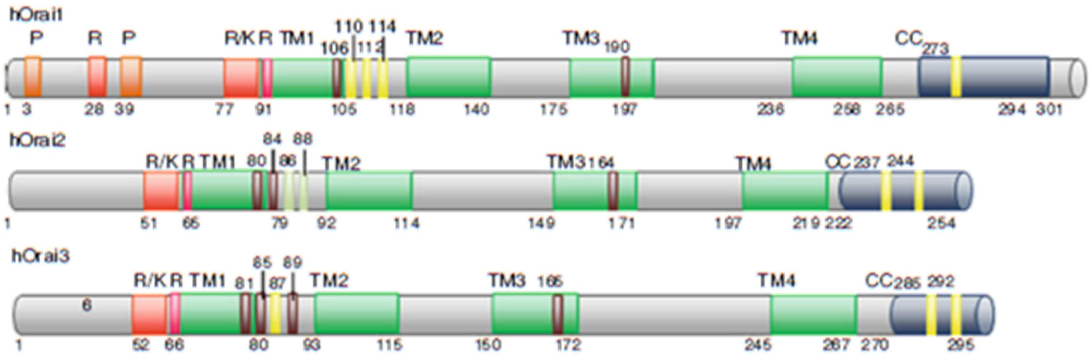
mRNA expression in normal tissues has been assessed by different techniques (microarrays, RNAseq, SAGE). Microarrays analyses show that ORAI3 is overexpressed in prostate, lung, monocytes and whole blood (http://biogps.org/#goto=genereportid=93129, with overexpression defined as 3 times the mean

expression observed in the 83 tissues or cells tested in this study). ORAI3 mRNA expression is least important in pancreas, brain (especially the occipital lobe) and T cells ($CD4^+$ as well as $CD8^+$).

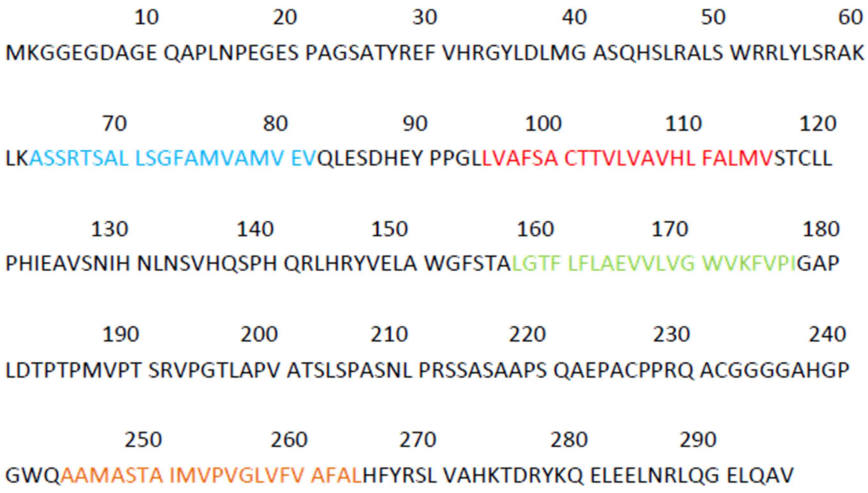
Protein

Description

Description of the protein sequence.
Molecular weight: 31499 Da.
Sequence length: 295 amino acids.
ORAI3 is a plasma membrane protein containing four transmembrane domains with intracellular N- and C-termini. ORAI3 contains a binding domain for calmodulin in its N-terminus, and a coiled-coil domain for protein interaction in its C-terminus.
Examination of the overall protein sequence of ORAI3 reveals high percentage of homology with the family members: 63.2% with ORAI1 and 66.4% with ORAI2 (60.3% between ORAI1 and ORAI2). These homology percentages increase when the comparison concerns the transmembrane domains: 93.8% with both ORAI1 and ORAI2, (92.5% between ORAI1 and ORAI2) (Feske et al., 2006; Hewavitharana et al., 2007). The pore-forming transmembrane domains of all three ORAI proteins show a high degree (~82%) of conservation.
The amino acid sequence of ORAI3 shows marked differences from its isoforms, particularly in the regions outside of the essential pore-forming domains, which might explain its unique properties and the differences with other isoforms in the modes of regulation and modulation from its isoforms (Shuttleworth, 2012).
The sequence identities between ORAI3 and ORAI1 in the cytosolic N- and C-termini are 34% and 46%, respectively, and is 21% in the extracellular loop between transmembrane domains 3 and 4 (Shuttleworth, 2012).
The N-terminus of ORAI3 comprises ~65 amino acids and has no clusters of prolines and arginines seen in ORAI1 (N-terminus domain containing ~90 amino acids and rich in clusters of prolines and arginines) (Takahashi et al., 2007; Frischauf et al., 2008). ORAI3 has a second extracellular loop linking transmembrane domains 3 and 4 which is longer than that of ORAI1 and ORAI2 (~72 amino acids in ORAI3 compared to only 38 amino acids in ORAI1). ORAI3 has a cluster of 22 positively charged amino acid residues immediately prior to the first transmembrane region which is fully conserved among all three ORAI channels (H44-R66 in ORAI3 and H69-R91 in ORAI1), and has three conserved glutamates located at the C-terminus to which is attributed the fast Ca^{2+}-dependent inactivation of ORAI3 (Lee et al., 2009).
The ORAI3 N-terminus appears critical for switching a store-operated channel to an exclusively arachidonate regulated channel (Thompson et al., 2010).
The residues E81 and E165 in the transmembrane domains 1 and 3, and E85, D87 and E89 in the extracellular 1-2 loop are critical determinants of a high Ca^{2+} selectivity. Other studies using a cysteine-scanning mutagenesis approach in ORAI3 revealed that Ca^{2+} selectivity was exclusively determined by the E81 residue alone (McNally et al., 2009).
Replacing the N-terminal cytosolic domain of ORAI3 with the corresponding domain of ORAI1 doubles the magnitude of the measured store-operated Ca^{2+} currents, whilst the reverse exchange virtually eliminates all currents. N-terminal deletion experiments narrow the critical region essential for the activation of ORAI3 to amino acids 42-62 (Lis et al., 2010). The appearance of significant store-operated currents dependes on a single specific lysine residue K60 in ORAI3, the conservation of this residue in ORAI1 and ORAI3 cannot explain the differences in the magnitude of store-operated Ca^{2+} currents between these two ORAI family members. N-terminal deletions of residues between W51 and Y55 significantly increase store-operated ORAI3-dependent currents (Bergsmann et al., 2011). The only sequence difference between ORAI1 and ORAI3 in this region is the substitution of a lysine in ORAI1 for an arginine at position 53 in ORAI3.



Schematic representation of ORAI protein structure and organization. Domains of human ORAI1, 2 and 3. P: proline-rich region, R: arginin-rich region, R/K: arginine-lysine-rich region, TM: transmembrane domain, CC: coiled-coil domain (Derler et al., 2012).



ORAI3 protein sequence of amino acids. ORAI3 protein (1 .. 295) has four helical transmembrane domains: T1 (63 .. 82) (20 amino acids), T2 (95 .. 115) (21 amino acids), T3 (157 .. 177) (21 amino acids), T4 (244 .. 264) (21 amino acids).

ORAI3 lacks C195, a reactive cysteine present in ORAI1 that serve as a detection system primarily for changes in the extracellular oxidative environment, and contains two additional cysteines within the extracellular loop between TM3 and TM4. The absence of C195 in ORAI3 makes it resistant to H_2O_2-inactivation, since pre-incubation with $H_2O_2$of ORAI1/STIM1 expressing cells (HEK; T cells) inhibits activation of ORAI1, but not of ORAI3, and reinsertion of C195 within ORAI3 renders ORAI3 channels redox sensitive (Bogeski et al., 2010).

Post-translational modifications of the protein

Glycosylation:

Unlike ORAI1, ORAI3 does not have a glycosylation site on the asparagine residue (N223) situated between the transmembrane domains TM3 et TM4 (Frischauf et al., 2008; Prakriya et al., 2006).

ORAI1 has a putative N-glycosylation motif (NVS) in its extracellular loop between predicted transmembrane segments 3 and 4. This motif is absent in ORAI2 and 3 (Gwack et al., 2007). ORAI3 migration properties do not change by tunicamycin treatment. Indeed, HEK293 cells stably transfected with FLAG-tagged ORAI and treated with 2µg/ml tunicamycin, showed that ORAI3 migrated at positions close to their predicted molecular masse (32.5 kDa).

Phosphorylation:

Since ORAI3 is a tetraspanning plasma membrane protein, it contains three intracellular regions that can potentially be phosphorylated by intracellular protein kinases: the N-terminus, an intracellular loop between transmembrane domains 2 and 3, and the C-terminus, each intracellular region potentially contains one or more phosphorylation sites. Ser-27 and Ser-30 have been identified as the main phosphorylation sites in ORAI1 within its N-terminus. They are conserved throughout evolution in all mammalian ORAI1 proteins. Mutations at these phosphorylation sites increase store-operated Ca^{2+} entry (SOCE) and CRAC current suggesting that ORAI1 phosphorylation at these residues by protein kinase C (PKC) suppresses SOCE and CRAC channel activation. However, Ser-27 and Ser-30 are not present in ORAI2 and ORAI3.

A phosphorylation of ORAI3 peptide has been revealed by a phosphoproteome analysis of human liver cells (Sui et al., 2008). This phosphorylation site is located in the C-terminus of ORAI3 on a tyrosine residue (Y278). Experimental ORAI3 phosphorylation has also been demonstrated in HEK293 cells (Kawasaki et al., 2010).

To examine in vivo PKC-mediated phosphorylation, HEK293 cells expressing FLAG-tagged ORAI were incubated with ^{32}P monosodium phosphate, and then stimulated with thapsigargin in the presence of extracellular Ca^{2+}. Thapsigargin mobilizes Ca^{2+} from the ER and the extracellular space and activates Ca^{2+}/DAG-dependent PKC isoforms. ORAI1 phosphorylation is enhanced in response to thapsigargin. The levels of ORAI3 phosphorylation have been less than half of that observed for ORAI1 (Kawasaki et al., 2010).

Other phosphorylation sites on ORAI3 were predicted by NetPhos2.0: 13 serine sites (S20, S45, S50, S57, S64, S65, S68, S86, S191, S203, S213, S214 and S20), 3 threonine sites (T26, T183 and T190) and 2 tyrosine sites (Y146 and Y278).

Expression

ORAI3 is only expressed in mammals (Cai, 2007). ORAI3 seems to be ubiquitously expressed in human (http://www.proteinatlas.org/ENSG00000175938/tissue), and mouse, showing a minor presence in skeletal muscle, spleen and colon (Cordeiro and

Strauss, 2011; Gao et al., 2010; Gross et al., 2007). More specifically, ORAI3 expression has been reported in brain, heart, kidney, testis, intestine, placenta, lung (Gwack et al., 2007; Motiani et al., 2013a), vascular smooth muscle cells (Trebak, 2012), airway smooth muscle in human (Peel et al., 2008) and macrophages. ORAI3 mRNA is usually much less expressed compared to ORAI1 in cells of lymphoid origin. ORAI1, ORAI2, and ORAI3 are expressed at similar levels in rat microglia (Hoth and Niemeyer, 2013).

Localisation

ORAI3 localizes to the plasma membrane and functions as a Ca^{2+}-selective ion channel (Feske et al., 2006; Vig et al., 2006; Zhang et al., 2006; Prakriya et al., 2006). This has been confirmed by immunocytochemistry of tagged proteins expressed in Jurkat T cells and in HEK293 cells. All three ORAI isoforms are expressed and localized at or near the plasma membrane, with little or no overlap with the ER marker ERP72. This localization was not grossly altered after store depletion with thapsigargin (Gwack et al., 2007). During meiosis, ORAI proteins get internalized into intracellular vesicles and store-operated currents are suppressed (Yu et al., 2009).

Function

In SOC channels:

ORAI3 presents a single putative channel pore and has a role as a store-operated Ca^{2+} (SOC) channel. SOC channels are the major route for Ca^{2+} entry in non-excitable cells, and they include ORAI channels characterized by high selectivity for Ca^{2+} over monovalent cations, low single-channel conductance (<1 pS), and an inwardly rectifying current-voltage (I-V) relationship. Functional CRAC/SOC channels are formed by a tetrameric assembly of ORAI1/2/3 subunits (Ji et al., 2008; Mignen et al., 2008a; Penna et al., 2008; Maruyama et al., 2009).

ORAI3 is different from its family members, notably because of its exclusive presence in mammals (Cai, 2007) and its receptivity to pharmacological modulation (Schindl et al., 2008). All three isoforms are selective to Ca^{2+}, ORAI3 being more permeant to monovalent cations such as Na^{+} (DeHaven et al., 2007). Indeed, the ORAI3 currents display a significantly increased permeability to Na^{+} when measured in the absence of external divalent cations (Lis et al., 2007).

ORAI3 expression is capable of inducing a store-induced conductance, but its magnitude is considerably smaller than that seen with ORAI1.

In HEK293 cells, human SCID T cells and fibroblasts, in which store depletion has been induced with thapsigargin, ORAI1 was shown to be the major regulator of store-operated Ca^{2+} influx, whereas ORAI3 can complement partially (partly compensate in the absence of functional ORAI1) and ORAI2 has a lesser role (Gwack et al., 2007). Combined overexpression of ORAI3 and STIM1 results in substantial reconstitution of Ca^{2+} entry in SCID fibroblasts (Gwack et al., 2007). ORAI3 expression also rescues normal store-operated Ca^{2+} entry in cells in which such entry was reduced by knockdown of ORAI1 (Mercer et al., 2006; DeHaven et al., 2007).

ORAI1, ORAI2, and ORAI3 channels are all similarly inhibited by extracellular Ca^{2+}, indicating similar affinities for Ca^{2+} within the selectivity filter. ORAI3 channels seem to differ from ORAI1 and ORAI2 in being somewhat resistant to the process of Ca^{2+} depotentiation (DeHaven et al., 2007). Moreover, like ORAI1, ORAI3 can potentiate store-operated Ca^{2+} entry in HEK293 cells expressing TRPC6 or TRPC3 (Liao et al., 2007).

ORAI3 and ORAI1 channels participate in store-operated Ca^{2+} influx in human airway smooth muscle cells (Peel et al., 2008). Cells transfected with siRNA against ORAI3 display abnormal (cyclopiazonic acid) CPA-mediated Ca^{2+} signals. Both Ca^{2+} release from the stores and Ca^{2+} influx are reduced in the ORAI3 knockdown cells, suggesting that cells with reduced ORAI3 expression have a lower Ca^{2+} store content and that ORAI3 plays a role in regulating basal Ca^{2+} levels or in Ca^{2+} release from the stores (Peel et al., 2008).

In addition, ORAI genes expression and CRAC activation has also reported in the human retinal pigment epithelium (Potier et al., 2009; Darbellay et al., 2009; Bisaillon et al., 2010).

ORAI3 upregulation contributes to vascular smooth muscle remodeling and neointimal hyperplasia caused by vascular injury.

ORAI3 has been shown to be an important component of store-independent arachidonate-regulated Ca^{2+} (ARC) entry in HEK293 cells (Mignen and Shuttleworth, 2000), and more recently of a store-independent leukotriene C4-regulated Ca^{2+} (LRC) entry pathway in vascular smooth muscle cells (Zhang et al., 2013).

In ARC channels:

ORAI3 has been identified as an essential component of the store-independent, arachidonic acid activated, Ca^{2+}-selective ARC channels (Mignen and Shuttleworth, 2000; Mignen et al., 2008b). These channels are found in a variety of different cell types, frequently co-existing with store-operated CRAC channels (Mignen et al., 2003; Mignen et al., 2005; Li et al., 2008; Yeung-Yam-Wah et al., 2010), and sharing similar basic biophysical properties. They are pentameric aggregates consisting of three ORAI1 and two ORAI3 subunits that form a functional ARC channel pore (Mignen et al., 2008b; Mignen et al.,

2007; Thompson et al., 2010). Two ORAI3 subunits are required within the pentamer to make the ARC channel sensitive to activation by low concentrations of arachidonic acid. ARC channels are characterized by being activated by low concentrations (2-8 μM) of arachidonic acid, insensitive to 2-APB, and with an absolutely dependence on the pool of STIM1 residing in the plasma membrane for their activation (Mignen et al., 2009). The acquisition of selective activation by arachidonic acid depends on the cytosolic N-terminal domain of ORAI3 (Thompson et al., 2010).

The ARC currents are distinguished from the co-existing CRAC channel currents by their store-independent activation, and the absence of any detectible fast inactivation. Expression of a dominant-negative mutant of ORAI3 (E81Q) had no effect on store-operated CRAC channel currents, but reduced currents through the store-independent ARC channels to negligible levels (Mignen et al., 2008b).

A recent study indicates a role of ARC channels in insulin secretion by pancreatic β cells (Yeung-Yam-Wah et al., 2010). It has been shown that the known ability of glucose and various insulin stimulants including acetylcholine and cholecystokinin to induce increases in cellular arachidonic acid results in activation of ARC channels in the β cells, increasing cytosolic Ca^{2+} levels and enhancing the subsequent insulin secretion (Yeung-Yam-Wah et al., 2010).

In LRC channels:

ORAI3 channels are also implicated in store-independent, leukotriene C4 (LTC4)-regulated Ca^{2+} (LRC) channels. Comparison of AA (arachidonic acid)- and LTC4-activated currents in vascular smooth muscle cells and in HEK293 cells using whole-cell and perforated patch-clamp recording shows indistinguishable non-additive LTC4- and AA-activated currents that both require ORAI1 and ORAI3. This suggests that ARC and LRC conductances are mediated by the same channel. Experiments using a non-metabolizable form of AA or an inhibitor of 5-lipooxygenase suggest that ARC and LRC currents in both cell types can be activated by either LTC4 or AA, with LTC4 being more potent. Although the plasma membrane (PM)-STIM1 was required for current activation by LTC4 and AA under whole-cell patch-clamp recordings in both cell types, ER-STIM1 was sufficient with perforated patch recordings. These results demonstrate that ARC and LRC currents are mediated by the same cellular populations of STIM1, ORAI1, and ORAI3 (Zhang et al., 2013).

In summary, ORAI3 proteins contribute to Ca^{2+} entry into cells through both store-dependent, Ca^{2+} release-activated Ca^{2+} (CRAC) channels and store-independent, arachidonic acid (AA)-regulated Ca^{2+} (ARC) and leukotriene C4 (LTC4)-regulated Ca^{2+}(LRC) channels (ORAI1/3 heteromultimers).

ORAI3 activation and interaction with STIM proteins

ORAI channels are activated by STIM1 or STIM2, single-pass transmembrane proteins localized predominantly in the membrane of the endoplasmic reticulum. STIM proteins have a long C-terminal cytoplasmic region and contain an N-terminal EF-hand located in the ER lumen that functions as a sensor of ER Ca^{2+} levels (Roos et al., 2005; Liou et al., 2005; Williams et al., 2001; Stathopulos et al., 2006).

The activation of ORAI channels by STIM depends on Ca^{2+} store depletion and is reversible once the stores are refilled (Luik et al., 2008; Soboloff et al., 2006). STIM1 activates store-operated Ca^{2+} channels only when it is not fixing Ca^{2+}, e.g. when the stores are depleted (Zhang et al., 2005). One minute after store depletion, STIM proteins are redistributed in puncta in close proximity to the plasma membrane (Liou et al., 2005; Luik et al., 2008; Várnai et al., 2007; Baba et al., 2006), where they co-localize with and activate ORAI channels, allowing Ca^{2+} influx (Liou et al., 2005; Wu et al., 2006; Muik et al., 2008). This process implies tetramerisation of STIM1 proteins using the N-terminus (Luik et al., 2008). It is thought that within these puncta, STIM1 communicates with and opens CRAC channels located to the plasma membrane (Luik et al., 2006; Parvez et al., 2008).

The initial interaction of STIM1 with the ORAI channels involves their cytosolic C-terminal region (Li et al., 2007; Muik et al., 2008; Frischauf et al., 2009). In all three ORAI subtypes, this region contains a predicted coiled-coil domain that is critical for interactions with STIM1 (Muik et al., 2008).

Truncation analysis identified a cytoplasmic region of STIM1, termed the CRAC activation domain (CAD)/STIM1 ORAI1 activating region (SOAR) to be sufficient to activate ORAI1 (Kawasaki et al., 2009; Muik et al., 2009; Park et al., 2009; Yuan et al., 2009). The cytoplasmic N and C termini of ORAI1 mediate channel opening by interaction with STIM1.

The activation of ORAI3-induced store-operated currents is significantly slower than that seen with ORAI1 and ORAI2 (Lis et al., 2007). Contrary to ORAI1, both ORAI2 and ORAI3 exhibit a 15-17 fold higher coiled-coil probability (Frischauf et al., 2009). A single point mutation in the ORAI1 coiled-coil domain (L273S) abrogates communication with STIM1 C-terminus (Frischauf et al., 2009; Muik et al., 2008). A single point mutation (L285S) within ORAI3 coiled-coil domain results in a partial inhibition of the interaction with STIM1 and subsequent activation of ORAI3 currents. Full inhibition of the ORAI3-

induced currents requires incorporation of an additional mutation (L292S) in the coiled-coil domain.

According to Bergsmann, activation of ORAI channels requires coupling of the C terminus of STIM to the N and C termini of ORAI (Bergsmann et al., 2011), since increasing N-terminal truncations causes a progressive decrease of ORAI3 fast inactivation concomitant with diminished binding to calmodulin.

Therefore, a fully conserved N-terminal ORAI region (aa 48-65 in ORAI3) is essential for STIM1-dependent STIMulation (Derler et al., 2009; Li et al., 2007; Yuan et al., 2009; Fahrner et al., 2009; Park et al., 2009; Lis et al., 2010).

Moreover, a single lysine within this conserved region (K60E in ORAI3) represents a critical residue for store-operated activation (Lis et al., 2010).

Interaction between ORAI family members

ORAI3 can multimerize with ORAI1 to form cation channels that conduct $Ca^{2+}$to some degree, since HEK293 cells stably expressing FLAG-tagged ORAI2 and ORAI3 revealed co-immunoprecipitation of ORAI2 and ORAI3 with transiently overexpressed Myc-ORAI1. Thus, ORAI members form homomultimers and can also form heteromultimers (Gwack et al., 2007).

Protein Interactions other than STIM

In addition to STIM1, p45 renamed as CRACR2A (CRAC regulator 2A) is also shown to co-immunoprecipitate with ORAI1, ORAI2 and ORAI3, suggesting a conserved binding mechanism with all the ORAI proteins, and that the ORAI channels, STIM1 and CRACR2A may form a ternary complex though direct interaction.

Various other proteins and lipids have been identified to interact with either STIM1 or ORAI3 or both. Among them is calmodulin (Mullins et al., 2009; Parvez et al., 2008; Bergsmann et al., 2011). Calmodulin binds to ORAI3 and, together with STIM, contributes to fast calcium-dependent inactivation; the structural studies show that CRACR2A/B is also able to interact with ORAI3 (Srikanth et al., 2010) but to date there is no evidence of functional regulation, because ORAI3 is able to form some complex with STIM-1 (Faouzi et al., 2011).

All proteins that interact with STIM1 are able to modulate ORAI3 function indirectly. Thus, SARAF (Palty et al., 2012), MS4A4B (Howie et al., 2009), Golli (Walsh et al., 2010), adenylyl cyclase type 8 (AC8) (Martin et al., 2009), the polycystin-1 cleavage product P100 (Woodward et al., 2010), caveolin (Yu et al., 2010), SPCA2 (Feng et al., 2010) and the L-type Ca^{2+}channel (Cav1.2) (Wang et al., 2010) or the phospholipids PIP2 and PIP3 (Korzeniowski et al., 2009; Walsh et al., 2009) are able to modulate indirectly ORAI3 activity.

ORAI3 inactivation

Fast inactivation of ORAI channels is mediated by cooperative interplay of several structures within ORAI proteins, by the CRAC modulatory domain (CMD) of STIM1, and via calmodulin binding to the ORAI N terminus (Parekh and Putney, 2005; Lee et al., 2009; Frischauf et al., 2011; Derler et al., 2009).

ORAI3 currents exhibit a marked fast inactivation within the first 100 ms, while that of ORAI2 or ORAI1 show less robust feedback regulation (Lis et al., 2007; Schindl et al., 2009; Lee et al., 2009). This effect depends on the presence of three conserved glutamates (E281, E283, E284) in the C-terminal region of ORAI3 (Lee et al., 2009). According to Yamashita et al. (2007), fast inactivation is determined by the same acidic residues involved in determining Ca^{2+} selectivity. A STIM1 C-terminus domain that include an acidic cluster (amino acids 475-483) termed CRAC Modulatory Domain (CMD) is also indispensable for fast ORAI channel inactivation (Derler et al., 2009; Mullins et al., 2009; Lee et al., 2009), since mutations in the CMD results in ORAI3 currents with attenuated or even abolished Ca^{2+}-dependent inactivation (Derler et al., 2009; Lee et al., 2009). On the other hand, Litjens et al. (2004) suggest that fast inactivation may be calmodulin (CaM) dependent and involves a region in the cytosolic N-terminal domain of ORAI3 (S45-K62) that binds CaM in a Ca^{2+}-dependent manner (Mullins et al., 2009; Frischauf et al., 2011). Transient CaM binding is assumed to mediate fast inactivation. The process may be that CaM transiently competes with STIM1 for the N-terminal interaction site on ORAI essential for channel gating.

Not only the C- but also the N-terminus and the second intracellular loop between TM2 and TM3 contribute to ORAI inactivation/gating in a cooperative manner (Frischauf et al., 2011) and modulate fast and slow inactivation as revealed by chimeric and mutational approaches (Srikanth et al., 2010). ORAI fast inactivation also involves the pore region since mutations of negatively charged residues within the pore of ORAI results in attenuation of Ca^{2+}-dependent inactivation (Yamashita et al., 2007).

Pharmacology

To date there is no specific inhibitor of ORAI3 but ORAI3 channels can be blocked by generic blockers of calcium entry channels such as La^{3+} (50-100 µM) and Gd^{3+} (1-5 µM). Other non-specific blockers include SKF96365, the myosin light chain kinase inhibitor ML-9 (Smyth et al., 2008), and the bistrifluoromethyl-pyrazole derivative BTP2 (Zitt et al., 2004) can be used.

Another compound extensively studied is 2-aminoethoxydiphenyl borate (2-APB), originally characterized as an inhibitor of $InsP_3$ receptors

(Maruyama et al., 1997; Bilmen and Michelangeli, 2002), later shown to have multiple diverse effects including both the inhibition and activation of various different members of the TRP channel family (Voets et al., 2001; Trebak et al., 2002; Chung et al., 2004; Hu et al., 2004; Li et al., 2006; Juvin et al., 2007), and the inhibition of SERCA pumps (Missiaen et al., 2001; Peppiatt et al., 2003), as well as to affect store-operated Ca^{2+} entry via CRAC channels (Gregory et al., 2001; Iwasaki et al., 2001; Prakriya and Lewis, 2001).

2-APB displays a bi-functional effect that is dependent on the concentration used. High concentrations of 2-APB were shown to increase store-operated currents in cells expressing STIM1 and ORAI3 (Lis et al., 2007; DeHaven et al., 2008; Peinelt et al., 2008; Schindl et al., 2008), accompanied by marked changes in ion selectivity by increasing ORAI3 channel pore size from ~3.8 Å to more than 5.34 Å, an effect that was apparently dependent on the E165 residue of ORAI3 that lies in the third transmembrane domain (Schindl et al., 2008). The residues that assist in formation of the 2-APB-activated ORAI3 pore are lined by TM1 residues, but also allows for TM3 E165 to approach the central axis of the channel that forms the conducting pathway, or pore (Amcheslavsky et al., 2014). Transmembrane domains 2 and 3, together with the linking intracellular loop, are required for 2-APB to directly activate ORAI3 channels (Zhang et al., 2008).

ORAI3 can be directly activated by high concentrations of 2-APB, in a STIM1- and store depletion-independent manner (DeHaven et al., 2008; Peinelt et al., 2008; Schindl et al., 2008; Zhang et al., 2008; Wang et al., 2009). These direct 2-APB induced currents display large inward and outward currents (i.e. they show double rectification) and a leftward shift in the reversal potential, features that indicate a marked reduction in Ca^{2+} selectivity, and an increased permeability to monovalent cations (DeHaven et al., 2008; Peinelt et al., 2008; Schindl et al., 2008; Zhang et al., 2008).

When ORAI3 forms a store operated channel, store-operated ORAI3 currents are potentiated by 2-APB at low concentrations (<10 μM) without affecting ion selectivity (Yamashita et al., 2011). This effect requires the presence of STIM1, and is strictly dependent on store depletion.

The most obvious unique property of the channels involving ORAI3 is their ability to be activated independently of store depletion, either pharmacologically by 2-APB or, physiologically, by agonist-generated increased levels of intracellular arachidonic acid.

A recent study by (Zeng et al., 2014) shows that the ryanodine receptor (RyR) agonist 4-chloro-3-ethylphenol (4-CEP) blocks ORAI1/3 store-operated channels. 4-CEP induces a significant Ca^{2+} release in rat L6 myoblasts, but inhibits SOCE. The inhibitory effect is concentration-dependent and more potent than the one of its analogues 4-CmC and 4-chlorophenol (4-ClP). In the HEK293 T-REx cells overexpressing STIM1/ORAI1-3, 4-CEP inhibited the ORAI1, ORAI2 and ORAI3 currents evoked by thapsigargin. The 2-APB-induced ORAI3 current was also blocked by 4-CEP. This inhibitory effect was reversible and independent of the Ca^{2+} release. The two analogues, 4-CmC and 4-ClP, also inhibited the ORAI1-3 channels. Excised patch and intracellular application of 4-CEP demonstrated that the action site was located extracellularly (Zeng et al., 2014).

GSK-7975A and GSK-5503A are selective CRAC channel blockers that inhibit both ORAI1 and ORAI3 currents by acting downstream of STIM1 oligomerization and STIM1/ORAI1 interaction, potentially via an allosteric effect on the selectivity filter of ORAI (Derler et al., 2012). Both GSK compounds fully inhibited ORAI3 currents. Similarly, Synta-66 inhibited ORAI3 currents at a similar rate as the GSK compounds. By contrast, 10 μM La^{3+} blocked ORAI3 currents more rapidly. The GSK compounds appeared to inhibit ORAI3 currents slightly faster than those of ORAI1. Overall these GSK compounds were equally effective at blocking ORAI1 and ORAI3, and inhibition occurred at a substantially slower rate than La^{3+}. Inhibition of ORAI currents by GSK compounds is not readily reversible: neither ORAI1 nor ORAI3 currents showed substantial recovery from block by GSK-7975A or GSK-5503A over a 4-5 min wash-out period.

2-APB stimulated ORAI3 currents are less susceptible to GSK-7975A. 10 μM GSK-7975A was totally ineffective in inhibiting these ORAI3 currents in contrast to those activated via STIM1. 50 μM GSK-7975A caused 50% inhibition and 100 μM GSK-7975A caused full inhibition. The GSK CRAC channel blockers did not differentiate between ORAI1 and ORAI3 channels consistent with the conserved pore geometry and selectivity filter among the ORAI isoforms.

Homology

ORAI3 (encoding gene: MGC13024 located on chromosome 16) has two human homologs: ORAI1 (FLJ14466, chromosome 12) and ORAI2 (C7orf19, chromosome 7) (Feske et al., 2006). ORAI3 made an evolutionary appearance in mammals, evolving from ORAI1 rather than ORAI2 (Cai, 2007) and manifesting conductances that display unique features in their gating, selectivity, regulation and mode of activation (Shuttleworth, 2012).

ORAI3 is the 'newest' ORAI family member in the evolutionary tree (Shuttleworth, 2012). Orthologous

ORAI3 genes are found in the following species: chimpanzee (98.98% homology), dog (92.20% homology), cow (90.51% homology), rat (89.83% homology), mice (88.48% homology).

Mutations

Note

Understanding of the role of ORAI1, and indeed its initial identification, came from the study of patients carrying functionally critical mutations in this gene. To date, no equivalent identification of patients bearing similar mutations in ORAI3 have been identified. (Diseases associated to absence of ORAI2, ORAI3 or STIM2 function have not been identified in human yet).

Implicated in

Note

ORAI3 overexpression is associated with breast, lung, leukemia and prostate cancers.

Breast cancer

Note

ORAI3 channels are reported to be highly expressed in breast cancer (BC) tissues and breast cancer cell lines MCF-7 and T47D compared to adjacent non cancerous tissues and non cancerous cell lines, respectively (Faouzi et al., 2011). They are also shown to be involved in proliferation, cell cycle progression and survival of BC cells by regulating the G1 phase and G1/S transition regulatory proteins. Thus, ORAI3 knockdown by specific siRNA inhibits cell proliferation, arrests cell cycle progression in G1 phase, and increases apoptosis in these cells (Faouzi et al., 2011). This phenotype is associated with a reduction in CDK4 and CDK2 (cyclin-dependent kinases) and cyclin E and cyclin D1 expression, an accumulation of p21Waf1/Cip1 (a cyclin-dependent kinase inhibitor) and p53 (a tumor-suppressor protein) together with an increase of Bax/Bcl-2 ratio. Interestingly, these effects seem to be specific to cancer cells, since down-regulation of ORAI3 channels does not affect either cell proliferation or cell survival of normal breast cells. Annexin V and 7-AAD double staining and analysis of the anti-apoptotic protein Bcl-2 to the pro-apoptotic protein Bax ratio revealed that the induced cell mortality by ORAI3 knockdown was mainly apoptotic as demonstrated by the increased percentage of Annexin V-positive cells and the increased Bax/Bcl-2.

The same study showed that ORAI3 contributes to Ca^{2+} influx in BC cells where both Store Operated Calcium Entry (SOCE) amplitude and resting $[Ca^{2+}]_i$ decreased significantly with ORAI3 knockdown. The authors concluded that the ORAI3 involvement in cell proliferation/survival and cell cycle progression may be at least partially linked to the calcium influx through the channels since the reduction of external calcium concentration $[Ca^{2+}]_o$ to 0.2 mM decreases significantly BC cell proliferation (Faouzi et al., 2011).

A subsequent study highlighted a correlation between ORAI3 and the oncogene c-myc expression in tumor tissues and in BC cell lines: ORAI3 and c-myc were over-expressed in 70% and 80% cases respectively. Expression of c-myc, as assessed by RT-qPCR, is higher in the MCF-7 cancer cell line than in the non-cancerous MCF-10A cell line. A similar over-expression pattern was shown for ORAI3 in these cell lines (Faouzi et al., 2013). ORAI3 down-regulation reduces both c-myc expression and activity levels exclusively in BC cells, whereas ORAI1 (one of the two mammalian homologs to ORAI3) induced an upregulation of c-myc mRNA. The involvement of c-myc in the ORAI3 signaling was demonstrated when silencing c-myc resulted in closely-similar and non-additive effects to the ones induced by ORAI3 downregulation: decreased cell proliferation, cell cycle arrest with a significant accumulation of the cells in the G0/G1 phase, increased cell mortality (Faouzi et al., 2013).

Authors showed that ORAI3 channels affect c-myc, most likely via the MAP Kinase pathway, as demonstrated by decreased phosphorylation levels of extracellular signal-regulated kinases 1 and 2 (ERK1/ERK2) after ORAI3 downregulation (Faouzi et al., 2013).

Parallel studies also reported that ORAI3 mediates SOCE in estrogen-receptor-positive (ER^+) BC cell lines (Motiani et al., 2010), whereas in estrogen-receptor-negative (ER^-) BC cell lines, SOCE is mediated by ORAI1. This study was the first to describe SOCE and endogenous calcium release-activated currents (CRAC) that are mediated by native ORAI3 channels and highlights a potential connection between estrogen receptor alpha (ERα) and ORAI3 (Motiani et al., 2010). Authors then reported that knockdown of ERα decreases ORAI3 expression level leading to a decrease in ORAI3-mediated SOCE and CRAC current, while activation of ERα increased ORAI3 expression and SOCE in MCF7 cells (Motiani et al., 2013b). Consistently with the above cited studies, ORAI3 knockdown inhibits SOCE-dependent phosphorylation of both ERK1/2 and focal adhesion kinase (FAK).

It also decreases the transcriptional activity of nuclear factor of activated T-cells (NFAT), which was associated with decreased cell growth and Matrigel invasion of ER^+ MCF7 cells in contrast to ER^- MDA-MB231 cells where no effects were observed (Motiani et al., 2013b).

Lung cancer

Note

An overexpression of ORAI3 was observed in 66.7% of human tumor samples as compared to the human non-tumoral samples (40/60) as revealed by immunohistochemistry. The 60 lung adenocarcinomas were classified according to grading system proposed by Yoshizawa et al. 2011 (low, intermediate and high grades). The ORAI3 staining score is reported to be highly expressed in higher tumor grade (high grade; n= 16) as compared to low tumor grades (Ay et al., 2013).

ORAI3 is also expressed in non small cell lung carcinoma cells (NSCLCC) such as NCI-H23, NCI-H460, A549 and Calu-1. In NCI-H23 and NCI-H460 cells, ORAI3 is a major actor of Store Operated Calcium Entry (Ay et al., 2013). Ay et al. (2013) demonstrated that ORAI3 is involved in NSCLCC proliferation. Indeed, ORAI3 inhibition induces a strong decrease in NSCLCC proliferation, accumulating cells in G0/G1 phase of the cell cycle. This accumulation in G0/G1 phase is associated with a decrease in Cyclin D1/cdk4 and Cyclin E/cdk2 proteins level. No effect is observed on apoptosis. The same study demonstrated that SOCE induces Akt phosphorylation in NSCLCC and ORAI3 inhibition decreases this activation demonstrating ORAI3 can promote proliferation through SOCE by activating Akt pathway. They also showed that neither ORAI1 nor ORAI2 are involved in SOCE in NSCLC cell lines, suggesting that ORAI3 is the main component of SOCE in those cells (Ay et al., 2013).

The same type of mechanism is observed with TRPC1 in NSCLCC. Indeed Tajeddine and Gailly (2012) have demonstrated that TRPC1 is involved in G1/S transition in A549 NSCLC cell line through SOCE. They showed that cell cycle arrest after TRPC1 inhibition induces a decrease in EGFR activation and subsequent signaling (PI3K/Akt, MAPK).

Those two studies suggest that SOCE is an important mechanism in proliferation of NSCLCC. Indeed, EGFR signaling is overactivated in NSCLCC either by constitutive activation of EGFR or K-Ras mutation. ORAI3, able to activate this pathway, hence can be a potential target for anti-cancer drug.

Myeloid leukemia

Note

The mRNA levels of ORAI3 in both human leukemia and human myeloma tipifarnib-sensitive cell lines were significantly higher than in the tipifarnib-insensitive human myeloma cells. Tipifarnib is a new apoptotic agent that inhibits farnesyltransferase responsible for the transfer of a farnesyl group to Ras protein. Tipifarnib activates ORAI3-mediated SOC leading to $[Ca^{2+}]_i$ increase. Moreover, ORAI3 functional expression was higher in 2-APB-sensitive leukemia and myeloid cells as compared to 2-APB-insensitive myeloid cells (Yanamadra et al., 2011). These results suggest that Tipifarnib-resistant cells express less ORAI3 ORAI3 conferring protection against apoptotic effect of Tipifarnib (Yanamadra et al., 2011).

Prostate cancer

Note

ORAI3 mRNA expression levels are significantly reduced in tumours when compared to non-tumour tissues from 13 prostate cancer patients. mRNA expression levels of ORAI3 are decreased in both androgen-sensitive human prostate adenocarcinoma cell line (LNCaP) and androgen-insensitive prostate cancer cell line (DU145), when compared to human prostate epithelial cells from healthy tissue. The pharmacological effects of 2-APB on CRAC channels in prostate cancer cells differ from those in human prostate epithelial cells, and siRNA based knock-down experiments indicate changed ORAI3 channel levels are underlying the altered pharmacological profile (Holzmann et al., 2013).

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