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Review

Focus on TRP channels in cystic fibrosis

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ABSTRACT

The Transient Receptor Potential (TRP) protein superfamily is a group of cation channels expressed in various cell types and involved in respiratory diseases such as cystic fibrosis (CF), the genetic disease caused by CF Transmembrane conductance Regulator (CFTR) mutations. In human airway epithelial cells, there is growing evidence for a functional link between CFTR and TRP channels. TRP channels contribute to transmitting extracellular signals into the cells and, in an indirect manner, to CFTR activity via a Ca^{2+} rise signaling. Indeed, mutated CFTR-epithelial cells are characterized by an increased Ca^{2+} influx and, on the opposite, by a decreased of magnesium influx, both being mediated by TRP channels. This increasing cellular Ca^{2+} triggers the activation of calcium-activated chloride channels (CaCC) or CFTR itself, via adenylyl cyclase, PKA and tyrosine kinases activation, but also leads to an exaltation of the inflammatory response. Another shortcoming in mutated CFTR-epithelial cells is a $[Mg^{2+}]_i$ decrease, associated with impaired TRPM7 functioning. This deregulation has to be taken into consideration in CF physiopathology, as Mg^{2+} is required for ATP hydrolysis and CFTR activity. The modulation of druggable TRP channels could supplement CF therapy either an anti-inflammatory drug or for CFTR potentiation, according to the balance between exacerbation and respite phases. The present paper focus on TRPA1, TRPC6, TRPM7, TRPV2, TRPV4, TRPV6 and ORAI 1, the proteins identified, for now, as dysfunctional channels, in CF cells.

1. Introduction

Cystic fibrosis (CF) is a genetic disease, due to CF Transmembrane conductance Regulator (CFTR) gene mutations, which is characterized by abnormal ion transport across the apical plasma membrane (PM) of epithelial tissues, including the airways [1]. CFTR is a plasma membrane protein that belongs to ATP binding cassette superfamily. It is a cAMP- and ATP-regulated channel which ensures, among others, Cl^- and bicarbonate transports. The most common CF mutation F508del-CFTR is the deletion of phenylalanine at position 508 leading to chloride impermeability in many exocrine glands (salivary, airways, pancreas) associated to reduced volume of the final secretory fluid [1–4]. Several studies described a non-intuitive consequence of CFTR mutation, which is important deregulation of Ca^{2+} homeostasis in CF cells. Ca^{2+} signaling deregulations were observed in several epithelial cell lines and also in primary epithelial cells [5,6]. This CF abnormal Ca^{2+} phenotype takes great importance in the CF physiopathology. First, CFTR protein expression is affected by an increased in intracellular $[Ca^{2+}]$ which down-regulate the CFTR mRNA level [7]. Second, the rise of intracellular $[Ca^{2+}]$ involves changes in immune and respiratory responses. It has been shown that Ca^{2+} entry in CF neutrophils, especially via TRP channels, is responsible for a reduced antimicrobial response [8]. An increased inflammatory response mediated by a rise in intracellular $[Ca^{2+}]$ signaling has also been reported in primary cells exposed to luminal inflammatory mediators [9]. Last, it has recently been shown that the increased $[Ca^{2+}]_i$ is involved in CFTR internalization [10].

Virtually every eukaryotic cell expresses at least some type of calcium channel, in the plasma membrane, in intracellular organelles, or typically both. Plasma membrane calcium channels are regulated by membrane voltage or by ligands, and in some cases by both. Not surprisingly, voltage-activated channels are generally encountered in cells that depend largely on excitable behavior, for example, muscle and nerve. Calcium channels that are activated by ligands are more broadly distributed but are the exclusive mediators of transmembrane calcium flux in non-excitable cells, for example, blood cells and epithelial cells. Among all the cationic channels involved in the physiology of non-excitable cells, the family of TRP (Transient Receptor Potential) channels is an important player in calcium homeostasis. TRP channels were discovered in the eye of the *Drosophila melanogaster* fly and named for their transient response to bright light. There are 28 mammalian TRP subunits, categorized into six related protein subfamilies, based on sequence homology [11]. TRP ion channels are widely expressed throughout the body and can respond to an important diversity of

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Table 1
Detection of TRP channel activity in the airway.

Lung cell	TRPA1	TRPC6	TRPM7	TRPV2	TRPV4	TRPV6
Epithelial cells	X	X	X		X	X
Macrophages		X		X		
Smooth muscle cells		X			X	
Neutrophils		X				
Alveoli					X	
References	[15]	[21,22]	[32]	[84]	[34,35,36,37,38]	[59]

Table 2
Comparison of TRP function in CF versus non-CF cells.

	Channels Function in CF vs non-CF	References
TRPA1	≈	[15]
TRPC6	↗	[22]
ORAI1-STIM1	↗	[28]
TRPM7	↘	[32]
TRPV2	↘	[84]
TRPV4	↘	[47]
TRPV6	↗	[59]

intracellular and extracellular stimuli. This ability to be activated by seemingly disparate mechanisms has led to the perception of TRP channels as multiple signal integrators. The TRP channel superfamily comprises a group of cation-selective proteins, which displays a general preference for calcium ions. Moreover, the TRP channel family has been implicated in the pathogenesis of relevant chronic respiratory diseases such as asthma, chronic obstructive pulmonary disease (COPD), chronic cough, nonallergic rhinitis or/and apneic responses [12].

The aim of this review is to describe the TRP channels, which have been explored in more depth regarding their role in physiological and pathological mechanisms in cystic fibrosis airway epithelial cells (TRPA1, TRPC6, TRPV4, TRPV6, and TRPM7).

2. TRPA1 channels

Human TRPA1 channels were first isolated from cultured fibroblasts [13] and originally named ANKTM1 due to a large number of ankyrin repeat domains. Only one homolog of the ankyrin subfamily has been identified in mammals. TRPA1 is a calcium-permeable non-selective cation channel belonging to the large TRP family of ion channels [14]. TRPA1 has important functions in the lung and upper airways (Table 1).

Prandini et al. [15] were the first to investigate the role of TRPA1 channels in CF. They found that TRPA1 channels modulate the inflammatory response of CF bronchial epithelia induced by exposure to planktonic bacterium or supernatant of mucopurulent material, resembling acute or chronic infection by *P. aeruginosa* [15]. Moreover, they showed that direct activation of TRPA1 with agonists is sufficient to induce the release of IL-8 in small airway epithelial cells [16]. Based on these observations, it was proposed that TRPA1 could represent a druggable target to control the excessive inflammation reported in the lungs of CF patients, reducing tissue damage without completely blunting the immune response. To support this hypothesis, it was shown that inhibition of TRPA1 calcium transport by selective antagonists and transient reduction of protein expression by gene silencing significantly reduces the transcription and release of different cytokines, including IL-8, IL-1β, and TNF-α [15]. Whether pharmacological inhibition of TRPA1 channels in airway epithelial cells could provide significant amelioration of CF lung inflammation remains yet unknown but it would be interesting to test new molecules able to target this channel, as TRPA1 blockers could improve respiratory disorders such as chronic cough, asthma, COPD and allergic rhinitis [17].

3. TRPC6 channels

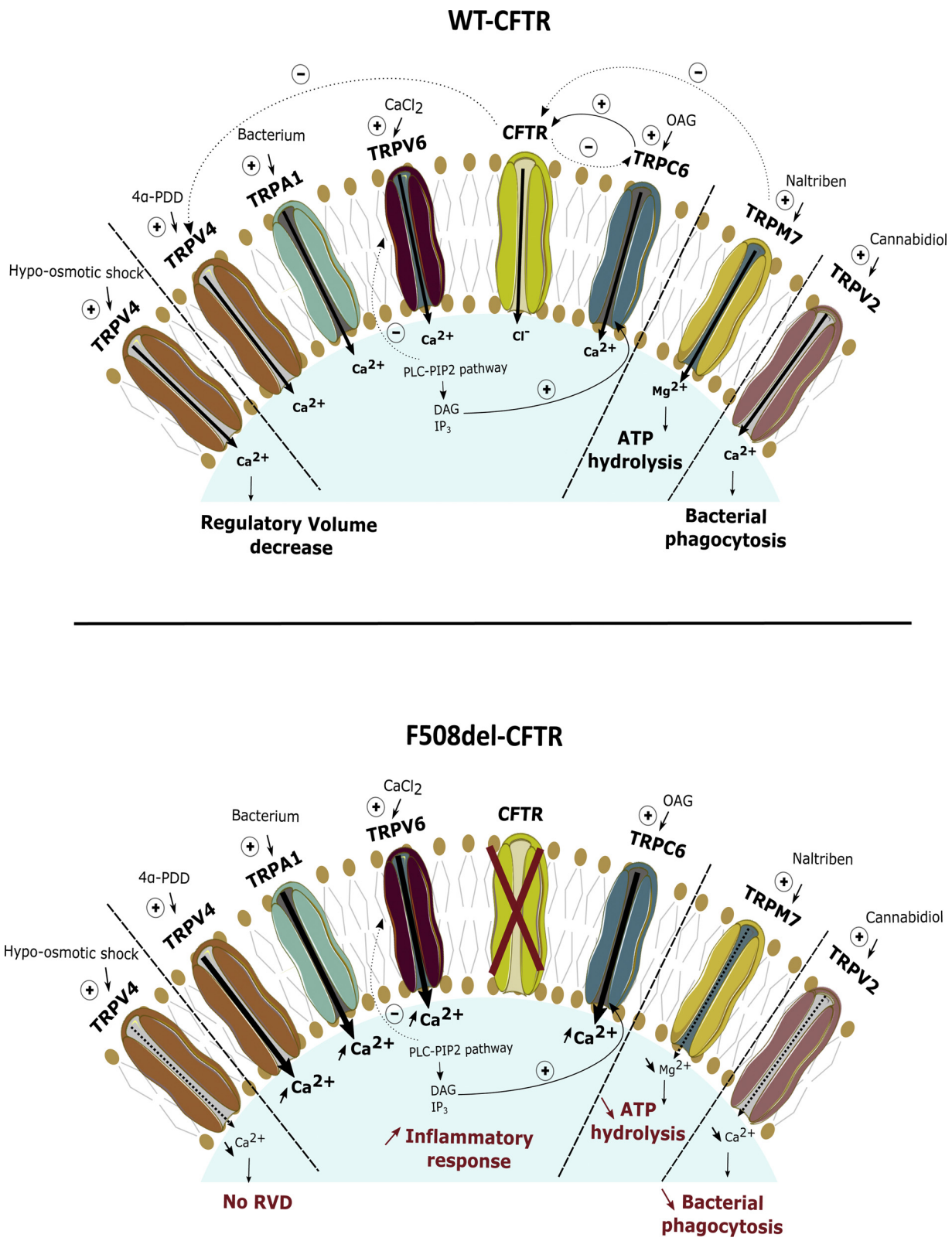
Human and mouse TRPC6 cDNAs were originally isolated from the pancreas [18] and it turns out that TRPC6 expression is the highest in the lung and brain [19]. In the context of respiratory diseases, TRPC6 channels appear to play a role in different cell types like neutrophils, macrophages and smooth muscle cells (reviewed in [20] and Table 1).

TRPC6 mRNA is also expressed in well-differentiated human bronchial epithelial cells [21] and TRPC6-mediated Ca^{2+} influx was reported to be significantly increased in CF human airway epithelial cells compared to non-CF cells when exposed to 1-oleoyl-2-acetyl-sn-glycerol (OAG), a membrane-permeable diacylglycerol analog that activates TRPC6 [22]. In CF cells expressing F508del-CFTR, specific silencing of TRPC6 reduced the abnormal Ca^{2+} influx response. Indeed, TRPC6-mediated Ca^{2+} influx, but not TRPC6 expression, is increased in CF cell lines expressing G551D-CFTR and F508delCFTR [22,23].

A correlation between abnormal Ca^{2+} homeostasis in CF cells and the expression of CFTR at the cell membrane was evidenced [23]. When CFTR is resident at the cell surface as it is the case for WT and rescued F508del-expressing CHO cells, abnormal Ca^{2+} influx induced by OAG is normalized [23]. Moreover, specific CFTR silencing in WT-CFTR expressing cells increases TRPC6-mediated Ca^{2+} influx [22], whereas CFTR activation by forskolin reduced by 30% the OAG-Ca^{2+} activity [23], showing that plasma-membrane activity of CFTR is required to down-regulate OAG-dependent Ca^{2+} influx. Antigny et al. related a direct link between TRPC6 and WTCFTR using co-immunoprecipitation studies, confirming this hypothesis. But it has also been shown that TRPC6 silencing diminished CFTR activity [22] and that OAG potentiates forskolin-dependent activation of CFTR [23] in WT-CFTR expressing cells. The authors stated a reciprocal functional coupling between these two channels in which CFTR down-regulates OAG-dependent-Ca^{2+} influx and this Ca^{2+} influx up-regulates CFTR-dependent Cl^- transport. Indeed, not only the upregulation of CFTR-WT activity was measured by enhancing OAG Ca^{2+} influx in non-CF cells, but in addition upregulation of G551D-CFTR activity was measured following activation of TRPC6 by OAG in the presence of the therapeutic agent VX-770 (Ivacaftor), an activator of G551D-CFTR [23]. In CF epithelial cells, guanabenz, an α2-selective adrenergic agonist, transiently increased $[Ca^{2+}]_i$ via an influx of the extracellular Ca^{2+} which stimulates CaCC [24]. TRPC6 channel is pivotal for the activation of CaCC by guanabenz through an α2-adrenergic-independent pathway suggesting a functional coupling between TRPC6 and CaCC channels [25]. A small molecule, (R)-roscovitine, (first identified in chemotherapeutics) rescues phagocytic function in CF pulmonary macrophages, independently of either kinase inhibition or CFTR expression [26]. The authors show that the TRPC6 calcium-permeable channel in the alveolar macrophages (AM) functions to shunt the transmembrane potential generated by proton pumping and is capable of restoring microbicidal function to compromised AMs in CF and enhancement of function in non-CF cells. TRPC6 channel activity is enhanced via translocation to the cell surface in response to G-protein signaling activated by (R)-roscovitine. These data show that enhancing vesicular insertion of the TRPC6 channel to the plasma membrane may represent a general mechanism for restoring phagosome activity in conditions, where it is lost or impaired.

4. The Orai1-STIM1-TRPC complexes

In addition to the family of TRPC channels involved in the Ca^{2+} influx into non-excitable cells, there are the ORAI channels. These channels are activated in response to cell stimulation and Ca^{2+} release from the endoplasmic reticulum (ER). The protein that conveys the Ca^{2+} content of the ER to the plasma membrane is the ER Ca^{2+} sensor



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protein stromal interaction1 (STIM1) (for review [27]). A study examined the novel hypothesis that store-operated calcium entry (SOCE) through Orai1 is abnormal in CF [28]. The authors suggest that F508del-CFTR cells have enhanced calcium entry which would be mediated by the ER-resident Ca^{2+} sensor STIM1 and the Ca^{2+} release-activated channel Orai1 [28]. They showed that either WT-CFTR or F508del-CFTR corrected is sufficient to restore SOCE. Indeed, PM expression of CFTR is the only way to regulate SOCE because the use of forskolin or CFTRinh172 did not affect this calcium increased. In fact, the elevated Ca^{2+} signaling is caused by an increase in the exocytotic insertion of Orai1 into the PM. The SOCE increased in CF cells enhances IL-8 secretion and may, therefore, contribute to the hyperinflammatory

Fig. 1. Overview of TRP regulation in CFTR-expressing cells suggests TRP as potential targets in cystic fibrosis. Calcium entry via TRPA1, TRPC6, TRPV4, and TRPV6 is extended in cystic fibrosis cells when stimulated with bacterium such as *P. aeruginosa*, OAG, 4α-PDD, and $CaCl_2$, respectively.TRPC6 is up-regulated by DAG, serine and tyrosine phosphorylation and phosphoinositides while TRPV6 is a constitutively activated channel regulated by $[Ca^{2+}]_i$ and PLC-PIP2 pathway. The rise of intracellular calcium is followed by the production of well-identified inflammatory mediators. On the other hand, calcium entry via TRPV4 in response to hypo-osmotic shock is decreased in CF cells. Moreover, calcium entry via TRPV2 in CF macrophages and magnesium entry via TRPM7 in CF cells is diminished when stimulated by cannabidiol or naltriben, respectively, compared to non CF cells. As magnesium is required for ATP hydrolysis, the consequence is a decrease of CFTR activity. This calcium raise and magnesium fall globally support the inflammatory state in cystic fibrosis cells. The different models used in these studies are summarized below: **TRPV4**: CF tracheal epithelial cell line CFT1 obtained from a CF F508del/F508del patient, HEK cell line and CFBE cell lines for the hypotonic shock response and mouse $CFTR^{+/+}$, mouse $CFTR^{-/-}$, bronchial NCI-H292 epithelial cell lines, non-CF MM39 epithelial cell line, CF-KM4 epithelial cell line for the 4α-PDD activation; **TRPA1**: Human A549 alveolar type II-derived epithelial cells, CuFi-1 transformed bronchial epithelial cell line from a CF patient with F508del/F508del genotype; **TRPV6**: CF human epithelial cell line CFBE41o-, non-CF human epithelial cell line 16HBE14o-, primary human airway epithelial cells from a CF patient (CF hAEC) and a non CF patient (hAEC); **TRPC6**: human non-CF epithelial cell line MM39, human CF epithelial cell line CF-MK4, CHO cell line over-expressing F508del-CFTR, G551D-CFTR or WT-CFTR; **TRPM7**: HeLa cell line overexpressing F508del-CFTR, G551D-CFTR or WT-CFTR; **TRPV2**: primary human macrophages from non-CF and CF patients.

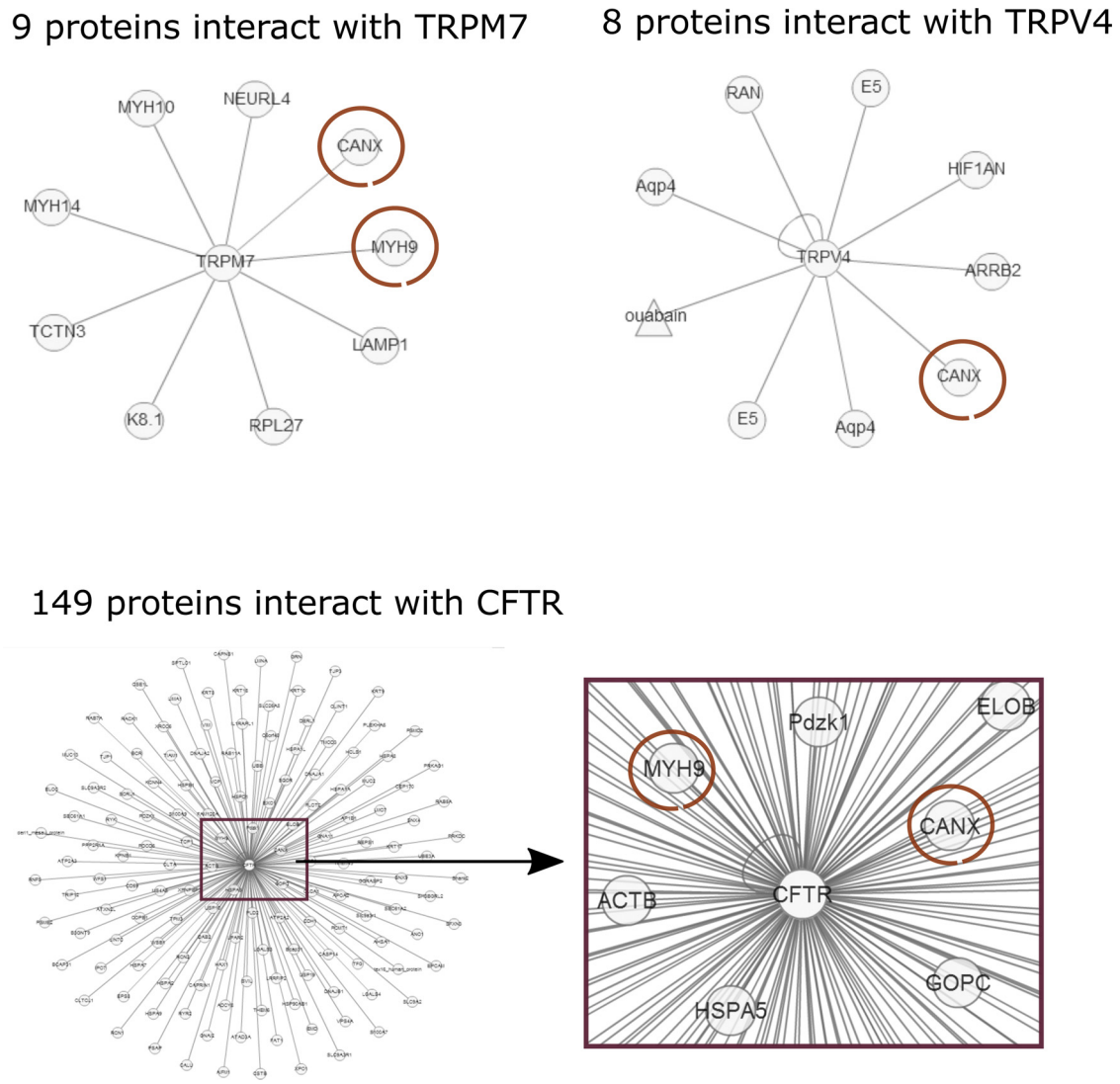

Fig. 2. Binary interactions reported with each TRPM7, TRPV4 and CFTR channels suggest a physical link between CFTR and TRPs. IntAct Molecular Interaction Database reported 9 protein interactions with TRPM7, 8 protein interactions with TRPV4 and 149 protein interactions with CFTR. Among the protein identified in complex with CFTR, myosin-9 is also interacting with TRPM7 and calnexin is linked both with TRPM7 and TRPV4.

state that characterizes CF [28].

5. TRPM7 channels

TRPM7 is remarkable in that it constitutes the fusion between a cation channel and a functional C-terminal serine/threonine-protein kinase domain (for review [29]). The tissue distribution of TRPM7 is ubiquitous, but expression levels in the human sample are the highest in heart, liver, bone and adipose tissue [30]. The TRPM7 channel is non-selective but predominantly permeates the divalent cations Ca^{2+} and Mg^{2+} under physiological conditions [31]. Consistent with its multi-functional nature, TRPM7 has been proposed to function in several fundamental physiological processes, including Mg^{2+} homeostasis, cell proliferation, cell motility and cellular differentiation (for review [29]).

TRPM7 altered expression or activity is associated with a number of pathological conditions, including cancer, cardiovascular defects and neurodegenerative diseases (for review [29]).

Huguet et al. [32] studied the expression, the function and the channel regulation of TRPM7 by $[Mg^{2+}]_i$ in non-transfected HeLa cells and in HeLa cells expressing WT-CFTR or mutated CFTR (F508del-CFTR and G551D-CFTR). In both F508del-CFTR and G551D-CFTR cells the protein expression of TRPM7 was increased whereas its function was reduced compared to WT-CFTR cells. Interestingly, Mg^{2+} had no effect on the channel regulation in these mutated cells, while it usually inhibits TRPM7 activity. As Mg^{2+} is required for CFTR activity through ATP hydrolysis, they measured $[Mg^{2+}]_i$ in cells expressing WT-CFTR, F508del-CFTR, and G551D-CFTR. They showed that $[Mg^{2+}]_i$ is decreased in cells expressing F508del-CFTR and G551D-CFTR. Then, in order to highlight a possible functional link between TRPM7 and CFTR, TRPM7 activity was pharmacologically increased by the use of naltriben, and the function of CFTR was studied in cells expressing WT-CFTR, F508del-CFTR, and G551D-CFTR. The authors found that the activation of TRPM7 by naltriben resulted in a decreased CFTR function in WT-CFTR and F508del-CFTR cells but in a highly increased function in cells expressing G551D-CFTR. Mg^{2+} and Ca^{2+} flux through TRPM7 were found to be modulated in cells expressing mutated CFTR compared to cells expressing WT-CFTR. The authors concluded that the link between TRPM7 and CFTR varies with the type of CFTR mutation and proposed naltriben as a new potentiator of G551D-CFTR, with potential implications for CF therapy [32].

6. TRPV4 channels

TRPV4 was originally isolated from rat kidney, and identified as a vertebrate homolog of the *Caenorhabditis elegans* gene *Osm-9*, with sequence homology similar to TRPV1 and TRPV2 channels [33]. TRPV4 is expressed in a wide range of tissues including heart, lung, kidney, DRG neurons, CNS, skin and sweat glands, with the most prominent expression found in epithelial and endothelial cells [33]. In the airways, TRPV4 channels are expressed in smooth muscles, alveolar wall, lung tissue and lung vessels [34–37], with the highest levels of expression in the epithelial linings trachea, bronchi, and lower airways, and in the alveolar septal walls [34,38,39] (Table 1). Interestingly, data is now emerging suggesting that there are genetic variants of TRPV4, which may be associated with the pathophysiology and symptomatology of asthma and COPD [40].

Human airway epithelia show a typical Ca^{2+}-dependent regulatory volume decrease (RVD) under hypotonic conditions [41], but this RVD response is lost in CF airways [42]. The cellular defect associated with the impaired RVD in CF epithelia is mainly linked to the dysfunction of volume-sensitive K^+ channels [42–45], although defective swelling-activated Cl^- channels have also been reported [46]. It has been proposed that the dysfunction of K^+ channels required for the RVD response might be related to the lack of a Ca^{2+} signal in CF epithelia, at least in the airway and small intestine epithelia [42,44,45]. Several studies by the group of Valverde demonstrated that the TRPV4 channel is the only pathway mediating the swelling-activated Ca^{2+} entry required to achieve a full RVD in human tracheal epithelial cells. Moreover, they have shown that the impaired RVD response in CF airway epithelia is caused by misregulation of TRPV4, suggesting that the hypotonic activation of TRPV4 channels is CFTR-dependent [47]. TRPV4 activation by hypotonic stress emerges as a key process in the control of cell volume in human airway epithelial cells, but it is impaired in CF airway epithelial cells, leading to defective RVD response [42,47]. The misregulation of TRPV4 channels may be relevant to the pathophysiology of CF in different ways. It might contribute to the increased Na^+ absorption described in CF airway epithelia [48]. Na^+ absorption is reduced by stimuli causing cytosolic Ca^{2+} increases [49]. Therefore, a defective TRPV4 channel might result in a reduced mechanically induced Ca^{2+} signal and in increased amiloride-sensitive Na^+ absorption. More recently, *in vitro* and *in vivo* evidence for an inflammatory role of TRPV4 in lung epithelium has been proposed [50]. The authors observed that four natural lipid-based TRPV4 agonists are present in expectorations of CF patients. Also, TRPV4-induced calcium mobilization and inflammatory responses were enhanced in CFTR-deficient cellular and animal models [50].

7. TRPV5 and TRPV6 channels

TRPV5 and TRPV6 constitute a distinct class of highly Ca^{2+}-selective channels within the TRP superfamily, which encompasses a diversity of non-voltage operated cation channels [51]. In humans, both channels are co-expressed in organs that mediate transcellular Ca^{2+} transport such as duodenum, jejunum, colon, and kidney, but also in the pancreas, prostate, mammary, sweat and salivary glands [51–53]. TRPV5 appears to be the major isoform in the kidney, whereas TRPV6 is more ubiquitously expressed with the highest concentrations in the prostate, stomach, brain, lung and small intestine. Ca^{2+}-selective TRPV6 channels are constitutively active [54] and play a critical role in calcium uptake in epithelial tissues [53,55]. TRPV6 KO mice exhibit disordered Ca^{2+} homeostasis, including defective intestinal Ca^{2+} absorption, increased urinary Ca^{2+} excretion, deficient weight gain, and reduced fertility, suggesting the pivotal role in Ca^{2+} homeostasis in tissues where this channel is expressed. TRPV6 is also expressed in epididymal epithelium where the protein was detected in the apical membrane [56]. The authors also found that male, but not female, mice homozygous for the mutation D541A on TRPV6 gene, which expressed a nonfunctional channel, showed severely impaired fertility. Altered TRPV6 expression is associated with a variety of human diseases [57], including cancers [58]. However, less is known about TRPV5 and TRPV6 expression and roles in airway epithelia.

TRPV5 and TRPV6 are both endogenously expressed in CF and non-CF epithelial cells [59] (Table 1). Compared to non-CF airway epithelial cells, the constitutive Ca^{2+} activity is increased two-fold in CF cells. Using pharmacological and siRNA strategies, it was demonstrated that TRPV6 is mostly responsible for this abnormal increase of Ca^{2+} influx. To further investigate the mechanism underlying this dysregulation, the authors focused on the negative feedback of phospholipase C (PLC)-PIP2 pathway on TRPV6 activity. Using PLC inhibitors they demonstrated that the decrease of PLC activity leads to an increase of constitutive Ca^{2+} influx in bronchial epithelial cells. Moreover, the diminished expression of the specific PLC-δ1 isoform confirmed the implication of the PLC-PIP2 pathway on TRPV6 regulation in bronchial epithelial cells. Interestingly, F508del-CFTR rescue by low temperature-induced a normalization of TRPV6 activity whereas the inhibition of TRPV6 had no impact on the activity of WT-CFTR and F508del-CFTR corrected or not. Moreover, PLC-δ1 protein expression level was not modified after F508del-CFTR correction, suggesting that the protein quantity is not the only factor that could explain the implication of this enzyme in the increase of TRPV6 activity cells [59]. The constitutive calcium influx is increased in CF cells, mostly due to the upregulation of TRPV6 activity. Vachel et al. [59] hypothesized that i) the decrease of the PLC-δ1 isoform expression contributes to dysregulation of TRPV6 channels in CF cells and that ii) PLC-δ1 protein intracellular localization may have an impact on the regulation of TRPV6 activity. The reduced expression of this intracellular mediator could have a larger influence by interfering with CFTR protein regulation and cytoskeletal organization in CF cells [59]. The abnormal Ca^{2+} influx through the TRPV6 channel could also participate in CF inflammation clinical features by increasing exacerbation via cytokine secretion.

8. Role of TRPs in the response of airway epithelial cells to environmental factors

Obstructive lung diseases such as CF and COPD are causes of high morbidity and mortality worldwide. CF is a multiorgan genetic disease

and is characterized by progressive chronic obstructive lung disease. Most cases of COPD are a result of noxious particles, mainly cigarette smoke but also other environmental pollutants. Although the pathogenesis and pathophysiology of CF and COPD differ, they do share key phenotypic features and because of these similarities, there is great interest in exploring common mechanisms and/or factors affected by CFTR mutations and environmental insults involved in COPD. In this context of as yet poorly understood molecular and cell signaling specificity leading to respiratory diseases, in recent years there has been a continuously growing interest to various members of TRP channels, many of which can rather specifically detect a diverse array of physical and chemical stimuli, such as mechanical forces, temperature changes, pH, osmolarity, noxious compounds, reactive oxygen and nitrogen species [60].

The cold-activated TRPA1 and TRPM8 and the heat-activated TRPV1 channels have received much interest in the studies of the respiratory system and its disease states [60]. These channels can modulate the action of thermal irritants, such as inhalation of cold air that can provoke coughing, especially in respiratory virus-induced cough hypersensitivity [61]. A recently identified association between six TRPV1 single nucleotide polymorphisms (SNP) and a higher risk for chronic cough in patients with enhanced susceptibility to cough [62] further strengthens the proposal that TRPV1 is a pathophysiological target for treating patients. However, it has also been reported that a loss of function single-point mutation of TRPV1 (TRPV1-I585V) produced a more severe cough phenotype [63]. Pharmacological studies showed that chemical irritants activate TRPA1 in the airways and produce asthma-like symptoms, as well as heightened responses to chemical and physical stimuli [64–67]. Pharmacological and knockout studies revealed that TRPA1 mediates the inflammatory effects of chemical irritants [68]. The expression and function of the cold- and menthol receptor TRPM8 in the airways has been addressed in several studies, but the data still remains somewhat controversial (see [66,69] for reviews). For example, the available pharmacological tools make it difficult to distinguish between the effects of TRPA1 or TRPM8 activation. It is, however, clear that rhinovirus upregulates expression of neuronal TRPM8 in a manner distinct from TRPV1 and TRPA1, and this implies TRPM8 contribution to virus-induced cough hypersensitivity [61]. Finally, *ex vivo* data, showing activation of vagal nerve fibers following TRPV4 stimulation [70] may represent the neurophysiological basis for the ability of the TRPV4 agonists to evoke sensory nerve firing, and the cough in a conscious guinea pig model [70]. However, which possibly endogenous stimulus might cause cough by targeting TRPV4 remains to be determined.

The environmental factors, as pollutants or smoke, are aggravating factors for CF patients, and then it could be interesting to study in-depth the role of these TRP channels in order to find new therapies and improve the care of these patients.

9. TRPs and airway smooth muscle dysfunction

The airways are built of the epithelium, smooth muscle, and connective tissue (fibroblasts), with an important contribution of sensory nerves and resident immune cells. The pulmonary vasculature consists of endothelial cells, smooth muscle, and connective tissue. TRP channels are expressed in all of these tissues and cells [71] and there is good evidence that they play a pivotal role in the development and maintenance of chronic lung diseases, ranging from chronic cough through COPD and asthma to idiopathic pulmonary fibrosis (IPF) [72].

Both exogenous and endogenous stimuli activate TRP channels, which include the release of inflammatory mediators and neuropeptides, contraction of smooth muscle, hypersecretion of mucus, and altered permeability; this eventually results in cough, chronic inflammation, lung injury, airway remodeling, and bronchoconstriction. TRPV1, TRPV4, and TRPA1 are all expressed in smooth muscle cells [73–75] and they are involved in bronchoconstriction, but via different pathways [76–78]. TRPV4-mediated bronchoconstriction is almost totally dependent on cysteinyl leukotrienes [79]. Activation of TRPV1 and TRPV4 induces contraction of ASM, but activation of TRPA1 by acrolein results in bronchial relaxation, reportedly via the release of PGE2 [80,81].

The involvement of some TRPs in ASM cells makes them new therapeutic targets for modulating bronchoconstriction in CF patients.

10. Role of TRPs in CF-affected tissues other than the airways

Cystic fibrosis is a disease of exocrine gland function that involves multiple organ systems but results in chronic respiratory infections, pancreatic enzyme insufficiency, and associated complications in untreated patients. Recent reports describe a fundamental role of TRP channels in intestinal epithelial cells in mediating cytokine/chemokine release as well [82]. Many TRP channels are expressed in various immune cells, especially in macrophages and T cells. Here, they modulate many functions such as cytokine expression and release, migration, or phagocytic activity. Moreover, in the epithelial layer, TRP channels expression was also found to be relevant in the pathogenesis of many inflammatory disorders, mainly through controlling chemokine/cytokine expression and release. Thus, a vital interplay between neurons, epithelial, and mucosal immune cells seems to maintain homeostasis in different organs, for example, the gut, the lung, and the vascular system and disruption of one or more of these players may induce disease (for review [83]).

In CF, macrophages have lost their capacity to act as suppressor cells and thus play a significant role in the initiating stages leading to chronic inflammation/infection. Levêque et al. [84] showed that *Pseudomonas. aeruginosa* recruits TRPV2 channels at the cell surface and induced a calcium influx required for bacterial phagocytosis. CF macrophages display perturbed calcium homeostasis due to a defect in TRPV2. In this context, deregulated TRPV2-signaling in CF macrophages could explain their defective phagocytosis capacity that contributes to the maintenance of chronic infection. Therefore, TRPV2 might be considered as a new target to restore phagocytosis capacity of CF macrophages and to improve the innate immune defense of patients with CF [84].

Patients with CF are highly susceptible to infections caused by opportunistic pathogens (including *Burkholderia cenocepacia*), which induce excessive lung inflammation and lead to the eventual loss of pulmonary function. Abundant neutrophil recruitment into the lung is a key characteristic of bacterial infections in CF patients. In response to infection, inflammatory neutrophils release reactive oxygen species and toxic proteins, leading to aggravated lung tissue damage in patients with CF. Robledo-Avila et al. [8] show a defect in reactive oxygen species production by CF neutrophils. Furthermore, dysregulated Ca^{2+} homeostasis led to increased intracellular concentrations of Ca^{2+} that correlated with significantly diminished NADPH oxidase response and impaired secretion of neutrophil extracellular traps in human CF neutrophils. Functionally deficient human CF neutrophils recovered their antimicrobial killing capacity following treatment with pharmacological inhibitors of Ca^{2+} channels and CFTR channel potentiators. TRPM2 and TRPM7 channels may regulate Ca^{2+} overloading in phagocytic cells [85,86], it is speculated that systemic pharmacological inhibition of these channels may help in restoring Ca^{2+} homeostasis and recovering the antimicrobial capacity in phagocytic cells [8]. This study suggests that regulation of neutrophil Ca^{2+} homeostasis (via CFTR potentiation or by the regulation of Ca^{2+} channels) can be used as a new therapeutic approach for reestablishing immune function in patients with CF.

11. Role of CFTR in TRP channels regulation: general findings

Although functional coupling has not been investigating yet between CFTR and all the TRPs, we can bring some general findings to answer why TRP channels are disturbed in CF cells (Table 2). First,

CFTR is known as a regulatory protein of several ion channels. It has been suggested that CFTR down-regulates ENaC [87–89] and CaCC [90,91] but up-regulates ROMK channels [92] and ORCC [93]. A functional link has been identified between WT-CFTR and two TRP channels as well (Fig. 1). Indeed, WT-CFTR down-regulates both TRPV4 and TRPV6 but with F508del-CFTR mutation, calcium fluxes mediated by these TRPs increase. In a different way, TRP channels can also regulate CFTR as it was experienced with TRPM7. In the presence of the TRPM7 activator naltriben, Huguet et al. [31] found lower activities of WT-CFTR and F508del-CFTR but a higher activity of G551D-CFTR, suggesting that TRPM7 activation generally down-regulates WT-CFTR in absence of cystic fibrosis disease. Second, differences in protein expression of TRP channels have been related with F508delCFTR mutation compared to WT-CFTR: Huguet et al. [32] noticed an increased TRPM7 expression in F508del-CFTR Hela cells whereas Levêque et al. [84] showed a decreased TRPV2 expression in CF macrophages from human patients. Balghi et al. [28] also revealed that F508del-CFTR mutation is associated with ORAI1 increased surface expression. It turns out that CFTR mutations clearly modify some protein channels expression. CFTR and TRPs, as membrane proteins, are using similar trafficking pathways from the ER to the plasma membrane after their synthesis [94]. They are both able to follow the constitutive exocytotic pathway or the regulated exocytotic pathway to reach the PM, through a host of protein-protein interactions [95]. Furthermore, CFTR and TRP channels both interact with SNAREs protein family, which allows vesicle targeting and fusion at PM. F508del-CFTR follows an unconventional trafficking/secretion mediated by proteins that differs from the conventional trafficking which concerns WT-CFTR channels [96,97].

Third, even though direct interactions between CFTR and TRP channels have not been identified yet, indirect interactions mediated by regulatory complex could be proposed. The interaction network between the seven TRP channels and CFTR was assessed with the online tool: IntAct Molecular Interaction Database (https://www.ebi.ac.uk/intact/) (Fig. 2). CFTR has many protein interactors, about 149 binary interactions have been reported until now, and CFTR interactome is studied with many interests [98,99]. The calcium-binding chaperone protein calnexin has been identified as a partner of TRPM7, TRPV4, and CFTR. Myosin-9 protein has been identified as a partner of TRPM7 and CFTR and has been related at the plasma membrane porosome complex of human airway Calu-3 cells [98].

As a matter of fact, we suppose that WT-CFTR indirectly controls TRP channels activity or expression in different ways, either per trafficking pathways and plasma membrane localization or per interacting proteins complexes. F508del-CFTR mutation possibly modifies cellular contents that usually connect CFTR and TRP channels.

12. Conclusions

Significant progress has been made in recent years in delineating the distribution and roles of TRP channels in various cell types under both physiological and pathophysiological conditions. It is already well established that TRP channels are important players in the pathogenesis of several human diseases, including in the respiratory field. The application of gene knockdown approaches and the recent development of selective pharmacological tools to probe TRP channel function [100] should promote our understanding and knowledge of the link between TRP isoforms and epithelial function and dysfunction in CF. Potential liabilities that may be associated with altering channel function might be discovered. Overall, in cystic fibrosis, TRP channels appear to involve an increase in intracellular Ca^{2+} and have consequences on the inflammatory response (Fig. 1). Moreover, the activity of some of these TRPs seems to influence or be influenced by CFTR. These channels can, therefore, become important therapeutic targets in this pathological context.

Conflict of interest statement

The authors reported no conflicts of interest.

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