KCNN4 Expression Is Elevated in Inflammatory Bowel Disease: This Might Be a Novel Marker and Therapeutic Option Targeting Potassium Channels

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ABSTRACT

Background & Aims: The K⁺ channel KCNN4 is involved in many inflammatory diseases. Previous work has shown that this channel is involved in epithelial ion transport and intestinal restitution. In inflammatory bowel diseases (IBD) a defective epithelial barrier can lead to typical symptoms like secretory diarrhea and the formation of intestinal ulcers. We compared surgical samples from patients with IBD, diverticulitis and controls without inflammation to determine the potential role of KCNN4 as a diagnostic marker and/or therapeutic target.

Methods: mRNA-levels of *KCNN4* and a control K⁺ channel were determined in intestinal epithelial cells (IEC) from patients with IBD, diverticulitis and controls. In addition, we performed a Western blot analysis of KCNN4 and a respective control K⁺ channel in IEC from patients with IBD. Furthermore, we determined epithelial barrier integrity by measuring the flux of fluorescent-labeled dextran beads across a cell monolayer upon incubation with interferon-γ.

Results: *KCNN4* mRNA and protein levels were elevated in IEC from patients with Crohn's disease (CD) and ulcerative colitis (UC). Of note, KCNN4 was not elevated in non-IBD intestinal inflammatory conditions e.g. diverticulitis. Of clinical relevance, pharmacological KCNN4 channel openers stabilized epithelial barrier function *in vitro*. Thus, KCNN4 may have a protective role in IBD and constitute a therapeutic target.

Conclusions: Our data demonstrate elevated KCNN4 both at mRNA and protein level in IEC specifically from patients with IBD. Therefore, we conclude that KCNN4 could be used as a novel marker for IBD, especially for the establishment of initial diagnosis. Of therapeutic consequence, we show that pharmacological KCNN4 openers stabilize the epithelial barrier. Thus, KCNN4 might be a novel target to diagnose and treat IBD.

Key words: KCNN4 - potassium channels - marker of IBD - Crohn's disease - ulcerative colitis – inflammatory bowel diseases.

Abbreviations: BCA: bicinchoninic acid; CD: Crohn's disease; EGF: epidermal growth factor; EGFR: EGF receptor; IBD: inflammatory bowel disease; IEC: intestinal epithelial cells; IFN-γ: interferon-γ; mRNA: messenger RNA; PCR: polymerase chain reaction; PI3K: phosphoinositide 3-kinase; PTPN2: protein-tyrosine phosphatase N2; Th: T helper; TNF: tumor necrosis factor; UC: ulcerative colitis.

INTRODUCTION

Despite many years of intensive research, the exact etiologies of the two entities of chronic inflammatory bowel diseases (IBD), Crohn's disease (CD) and ulcerative colitis (UC), remain unclear. Current hypotheses suggest that genetically predisposed individuals develop an exaggerated response to bacterial components in the gastrointestinal tract, translating to an excessive immune reaction towards the commensal flora. The etiology is multifactorial, and many gene loci have been found to serve as susceptibility genes for different subgroups of patients with IBD [1, 2]. Environmental influences are also considered as factors of IBD onset. Recent data highlight the role of intestinal flora composition in both initiation and propagation of chronic inflammation [3, 4]. In CD a predominant T helper 1 (Th1)-immune response has been described, whereas in UC a predominant T helper 2 (Th2)-immune response has been identified [5]. Interferon-γ (IFN-γ) plays a pivotal role in different models of murine colitis, especially in the commonly used dextran sulfate sodium-induced murine colitis [6] as well as in CD, where IFN-γ levels are elevated in

serum and mucosa [7, 8]. IFN-γ and other proinflammatory cytokines such as the tumor necrosis factor (TNF) can disrupt tight junctions and cause epithelial damage leading to a leaky mucosal barrier [9]. Persistent levels of inflammatory cytokines mediate impairment of tight junction and apoptosis of intestinal epithelial cells (IEC) [10], which increase epithelial permeability [11]. The epithelial barrier function in the intestinal tract is not only essential for regulated ion transport processes of the epithelium, but also for wound healing. During inflammation, superficial wounds occur constantly and are quickly restored by intestinal epithelial restitution [12]. This process is supported by different growth factors, including epidermal growth factor (EGF) that promotes epithelial migration of intestinal cells [13].

Our research group [14] and others [10] investigated the role of the intestinal epithelial monolayer in chronic intestinal inflammation with overproduction of IFN-γ. We described a defective EGF receptor (EGFR) activation in IEC with a shift towards the phosphoinositide 3-kinase (PI3K) pathway caused by IFN-γ [14].

Alterations in downstream signaling pathways originating at EGFR play a pivotal role for ion transport responses as well as for intestinal restitution. Preincubation of IEC with IFN-γ exerts a direct influence on anion secretion by abrogating the inhibitory effect of EGF on carbachol-induced K⁺ currents [15]. These data demonstrate that Ca²⁺-dependent K⁺ currents are altered in intestinal inflammation when elevated IFN-γ levels are present. Basolateral K⁺ currents are the driving force for apical sodium secretion [16, 17]. KCNN4 is a predominantly basolateral located Ca²⁺-activated K⁺ channel that is not only involved in ion transport responses in inflammation, but also in epithelial restitution [12, 13, 18, 19]. The epithelial barrier function maintains electrical gradients necessary for controlled regulation of absorption vs. secretion of electrolytes and water in the healthy intestine. A disturbed balance between absorption and secretion of the intestinal epithelium can lead to secretory diarrhea, one of the major symptoms of both CD and UC. In earlier studies, we observed a disruption of the actin cytoskeleton after prolonged IFN-γ treatment of the epithelial cell monolayer [14, 20]. This may constitute an alternative mode of action of IFN-γ in addition to the disruption of tight junctions. Of note and of clinical relevance, both mechanisms lead to a leaky epithelial barrier.

A defective epithelial barrier with enhanced permeability of the intestinal epithelium seems to be involved in the development of diarrheal symptoms primarily in CD. Further data indicate that a reduced K⁺ conductance in UC leads to reduced sodium absorption that could account for diarrheal symptoms [21]. In addition, defects in epithelial integrity together with an excessive immune response to the commensal flora may initiate chronic intestinal inflammation and trigger the onset of UC and CD [22]. Proinflammatory cytokines, predominantly IFN-γ, are elevated in the mucosa of CD patients. Several genes involved in the development of IBD seem to influence permeability of the epithelial barrier in conjunction with IFN-γ secretion [23]. In an earlier study we investigated the role of the CD candidate gene protein-tyrosine phosphatase N2 (PTPN2) in maintaining the epithelial barrier function after preincubation with IFN-γ. PTPN2 plays

a protective role by reducing IFN-γ-induced signaling and consequent induction of barrier defects [24]. Furthermore, earlier studies from our group revealed a prominent role of KCNN4 in Ca²⁺-dependent ion transport and intestinal restitution [14]. In a T cell-mediated mouse model of colitis, inhibition of KCNN4 ameliorated the disease [24]. Increased epithelial permeability has been described as an important pathophysiological mechanism for IBD [21]. Defective tight junctional complexes mediate dysfunctions of the epithelial barrier. It has been shown that K⁺ channels have an impact on tight junctions in epithelial cells [25, 26]. However, the role of KCNN4 in this network has not been investigated. It is known that K⁺ channels are involved in intestinal restitution.

We have previously demonstrated that the inhibition of the Ca²⁺-activated K⁺ channel KCNN4 differentially regulates IEC migration in inflamed vs. non-inflamed conditions and that migration is mediated by the PI3K signaling pathway [14]. The apical and basolateral K⁺ channel KCNN4 has been shown to be involved in different inflammatory diseases e.g. IBD and chronic inflammatory airway disease [26, 27]. KCNN4 is able to enhance the proliferative response of mesenteric lymph nodes in an experimental mouse model of colitis [28]. The basolateral cAMP-dependent K⁺ channel KCNQ1 is abundant in the intestine and was proven to be essential for intestinal chloride transport [29]. Accordingly, K⁺ channel KCNQ1 is a suitable control to further characterize the Ca²⁺-activated K⁺ channel KCNN4 in IEC. K⁺ channels interact with growth factor-dependent signaling cascades during intestinal epithelial restitution. Elevated K⁺ channel expression has been shown in different cell types in the mucosa of patients with ileal CD [30]. However, the clinical relevance of this finding has not been analyzed so far. Thus, we aimed to analyze the expression of KCNN4 at mRNA and protein level in IEC isolated from patients with IBD compared to controls in order to investigate whether this channel could serve as a potential clinical marker for IBD. Since we had the opportunity to analyze intestinal tissue specimens from bowel resections, we were able to isolate and analyze primary IEC from a cohort of 60 patients with CD and UC regarding *KCNN4* expression. In addition, we investigated whether KCNN4 constitutes a novel potential therapeutic target by monitoring the impact of channel inhibitors and openers on epithelial barrier integrity.

METHODS

Cell culture
The newborn rat non-transformed IEC line IEC-18 (ATCC CRL-1589; passages 25-55) was purchased from ATCC (Manassas, VA, USA). The IEC-18 cell line is derived from ileal crypt cells [31]. IEC-18 cells were cultured in DMEM supplemented with 4.5 g/l glucose, L-glutamine (PAA, Pasching, Austria), 5% FCS (Sigma Aldrich, Steinheim, Germany), 100 U/ml penicillin, 100 µg/ml streptomycin (PAA, Pasching, Austria) and 0.7 mM bovine insulin (Sigma-Aldrich, Steinheim, Germany) in a humidified atmosphere at 37°C with 10% CO2 [32, 33]. Sub-cultivation of IEC-18 was carried out twice per week in a ratio of 1:6. Fully supplemented cell culture medium was routinely filtered through steriflip filters (Millipore, Billerica, MA, USA).

Isolation of primary intestinal epithelial cells

We obtained 60 surgical specimens from patients with CD, UC, sigmoid diverticulitis and colorectal carcinoma neoplasia (> 10 cm distance from the tumor) after written informed consent. The Ethics Commission at the University of Regensburg had accepted a specific request (record number 00/14) and the procedure was performed according to the Declaration of Helsinki. Intestinal epithelial cells were isolated as previously described [34, 35].

RNA isolation and real-time-PCR Analysis

We used the RNeasy Mini Kit (Qiagen®, Hilden, Germany) to isolate RNA according to the manufacturer's instructions. cDNA was synthesized using affinity script reagents (Agilent Technologies GmbH, Böblingen, Germany) as given in the manufacturer's protocol. Real-time-PCR was executed by using 2x Brilliant® II Mastermix qPCRHigh ROX (Agilent Technologies GmbH, Böblingen, Germany) and Human GAPDH-Mix (20x) (Life Technologies, Carlsbad, USA) with Light Cycler®480 (Roche Diagnostics Deutschland GmbH, Mannheim, Germany) according to the manufacturer's specifications.

Primer sequences were as follows: human KCNN4 forward 5`-CCC TCA TCA AAA ACA CTC TCA CTA TG-3`, reverse 5`- TCC AGT CGC CTG CAC TTG-3`, probe 5`- FAM-TGC TAT GGA CGA CCT CCA GCT CTC AGT T-TAMRA-3`, human KCNQ1 forward 5`- CGC ATG GAG GTG CTA TGC T-3`, reverse 5`- GGC CTT CCG GAT GTA GAT CTT-3`, probe 5`- FAM-AGA ACC CCG ACT CCT CCA CCT-TAMRA-3` (all from Eurofins MWG Operon, Ebersberg, Germany).

Human GAPDH mix was used as endogenous control (Applied Biosystems, Carlsbad, CA, USA). PCR conditions were two minutes of incubation at 50°C, followed by ten minutes at 95°C and 40 cycles for 15 seconds each at 95°C for denaturation and 60 seconds at 60°C for annealing and extension each. Analysis of the results was performed by the delta-delta cycle threshold (ddCT) method [36].

Western Blot analysis

Primary IEC were isolated as described above. After removal of the crypts, cell pellets were resuspended in 300-750μl RIPA-buffer (Sigma Aldrich Chemie GmbH, Steinheim, Germany). The suspension was centrifuged at 13000 rpm, the clear supernatant was collected and stored at -20°C. BCA (bicinchoninic acid) assay was used for measuring protein concentrations.

Volumes containing 7.5 μg protein each were loaded on a 4-12% polyacrylamide gel to separate proteins by electrophoresis. Proteins were then transferred onto nitrocellulose membranes. NuPage instruments and reagents were used according to the manufacturer's instructions (all from Invitrogen, Carlsbad, CA). Membranes were blocked with 5% BSA (Biomol, Hamburg, Germany) in a Tris-buffered saline with 0.1% Tween 20 (TBST) (Sigma-Aldrich, St Louis, MO, USA) overnight, followed by incubation with the respective primary antibody diluted in blocking buffer: rabbit anti-KCNN4 polyclonal antibody (Bioss, Woburn, MA,

USA) 1:500 or rabbit anti-KCNQ1 polyclonal antibody (Bioss, Woburn, MA, USA) 1:200 and for loading control anti-ß-actin (Sigma Aldrich Chemie GmbH, Steinheim, Germany). As a secondary antibody we used anti-rabbit-IgG-HRP (Santa Cruz Biotechnology, Heidelberg, Germany) (1:3000 in 10 ml 2.5% milk/2.5% BSA). Membranes were exposed to 5 ml of enhanced chemiluminescence (ECL)-solution (2.5 mM luminol, 0.1 M Tris-HCl and 0.4 mM p-coumarin acid – all from Sigma-Aldrich Chemie GmbH, Steinheim, Germany – in aqua dest.) mixed with 1.53 μl of 30% hydrogen peroxide (Merck, Darmstadt, Germany) for five minutes. Detection of immunoreactive proteins was performed by using a Biorad Imager and Image Lab™ Software. For multiple use, membranes were stripped using ReBlot-plus strong solution (Chemicon, Temecula, CA, USA).

Permeability assay

Transepithelial permeability was assessed by measuring fluorescein isothiocyanate-dextran (FITC-dextran, 40,000 mmol, Sigma Aldrich Chemie GmbH, Steinheim, Germany) flux across IEC-18 cell monolayers. 200,000 cells per well were seeded into Millicell Cell Culture Inserts (Merck Millipore GmbH, Billerica, MA, USA), which were placed in the wells of a 24-well plate (Costar Corning Incorporated, Corning, NY, USA). IEC monolayers were preincubated for 24 and 48 hours with IFN-γ. As pharmacological K$^+$ channel modulators we used the KCNN4 opener SKA-31 at a concentration of 5 μM [37] as well as the KCNN4 opener 1-EBIO at a concentration of 600 μM [38]. Moreover, we used the following KCNN4 inhibitors: TRAM-34 (25 μM) [39, 40] and clotrimazole (10 μM) [39]. Clotrimazole is an FDA-approved KCNN4 channel blocker and cytochrome P450 inhibitor. TRAM-34 is an analogue of clotrimazole which has no impact on cytochrome P450 [41]. In addition, the KCNMA1 blocker iberiotoxin (100 nM) was used to examine possible unspecific effects modulated by other K$^+$ channels [42, 43].

After the respective treatment, IEC-18 cells were washed three times with Ringer's solution. A dilution (1.25 mg/ml) of FITC-dextran was mixed and 100 μl of this solution were added to the apical side of the monolayer in each insert [44, 45]. After incubation periods of 30 minutes and 1 hour, 100 μl of the basolateral solution was removed and fluorescence was measured using a Tecan Spectra Fluor fluorescence and absorbance reader (Tecan Group AG, Männedorf, Switzerland). Molecules were excited at 485 nm and the emission was measured at 535 nm.

Statistical analysis

For statistical analysis and creating diagrams IBM SPSS Statistics 20 was used. The Kolmogorov-Smirnov-test was performed for normal distribution and the Mann-Whitney-U-test was applied for calculation of p-values. Statistical p-values of 0.05 or less were considered to be significant. Outliers were eliminated after performing the outlier test by Grubbs.

Data analyzing protein levels are shown as x-fold expression relative to the mean of the control group.

RESULTS

mRNA levels of potassium channel *KCNN4* in intestinal epithelial cells isolated from surgical specimens of patients with IBD

To analyze the clinical significance of the Ca^{2+}-activated K^+ channel KCNN4 in human IBD, we isolated IEC from surgical specimens of patients with IBD (and other disease entities as controls) and quantified the expression of *KCNN4*. In total, we analyzed mRNA data from IEC of 60 patients. Subgroups were composed of specimens from patients with CD (n=28), UC (n=14), colorectal carcinoma (n=10) and diverticulitis (n=8). Table I provides an overview of the patients enrolled in our study. In macroscopically inflamed tissue from UC (n=14) and CD (n=28) patients, *KCNN4* expression in IEC was increased compared to respective controls from colorectal carcinoma without macroscopically detected inflammation (n=10). *KCNN4* transcription was significantly upregulated in UC (mean 658.2±158.7%; n=14) compared to colorectal carcinoma (0±37,8%; n=10) as well as in CD (415.6±124.1%) compared to colorectal carcinoma (Fig. 1A). In diverticulitis (662.7±511.8%) no significant differences were observed, neither in comparison to colorectal carcinoma nor in comparison to CD nor to UC (Fig. 1A). Expression of *KCNQ1*, a voltage-gated K^+ channel, was used as control. Levels of *KCNQ1* mRNA were comparable in all groups tested (Fig. 1B). There was no significant change observed in UC (-24.65±136.6%) compared to colorectal carcinoma (p=0.212), in CD (21.5±23.9%) compared to colorectal carcinoma (p=0.708) and in diverticulitis (256.4±176.5%) compared to colorectal carcinoma (p=0.762) (Fig. 1B). Therefore, a general effect on K^+ channel expression mediated by inflammatory responses is unlikely.

KCNN4 protein level in patients with IBD

We investigated the KCNN4 protein content in IEC from patients with IBD (n=6), diverticulitis (n=4) and controls with colorectal carcinoma (n=3). To distinguish between inflamed and non-inflamed conditions we compared all patients with intestinal inflammation (UC, CD and diverticulitis) to controls without inflammation (colorectal carcinoma). Of clinical relevance, we could show a significant upregulation of KCNN4 protein content in IEC from patients with intestinal inflammation (63.8±22.0%; n=10) compared to controls (0±5.5%; n=3) (Figs. 2A und 2B). The content of control protein KCNQ1 was not altered in all tested groups (2.8±5.0%; n=10) (Figs. 3A and 3B).
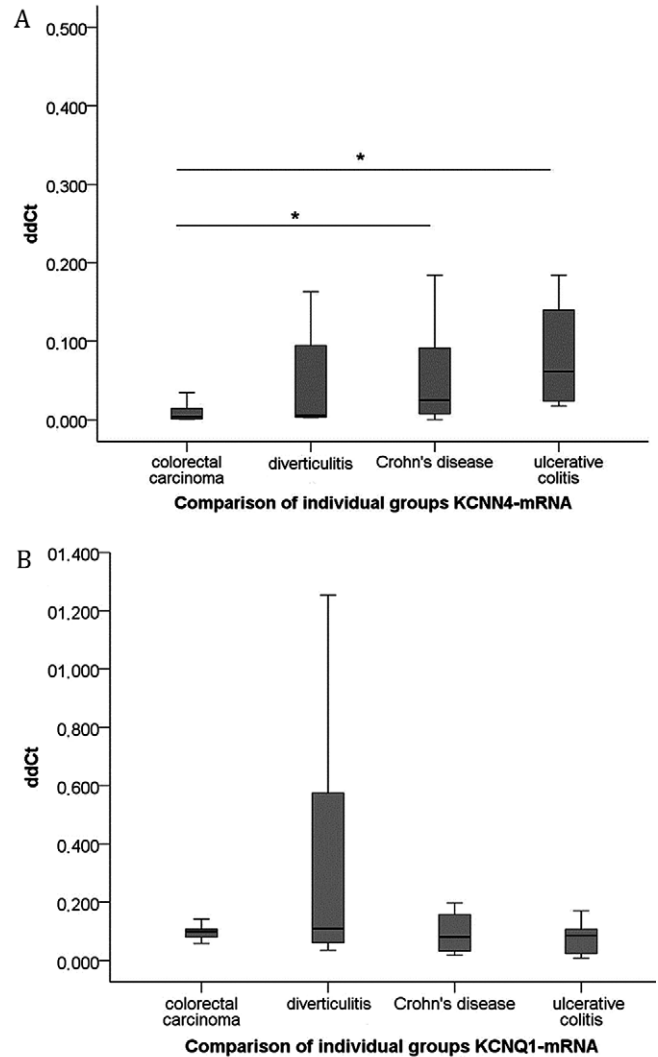

Fig. 1. *KCNN4* mRNA levels are elevated in IBD compared to controls. A) *KCNN4* mRNA expression, shown as absolute ddCt values (all performed in triplicate), was analyzed in IEC from patients with CD (n= 28), UC (n= 14), diverticulitis (n=8) and controls (colorectal carcinoma) (n=10). B) *KCNQ1* mRNA expression, shown as absolute ddCT values (all performed in triplicate) in IEC from the same patient groups. Data are expressed as -fold control. Asterisks denote statistically significant differences from control (*, p ≤ 0.05).

Targeting of KCNN4 by potassium channel openers on epithelial barrier function

Monolayers of IEC-18 cells were preincubated for 24 and 48 hours with IFN-γ. To analyze permeability, we measured

Table I. Patients characteristics

Patients characteristics		Diagnosis			
		Ulcerative colitis	Crohn's disease	Sigmoid diverticulitis	Colorectal carcinoma
Samples of patients, N (%)	60 (100.0%)	14 (23.3%)	28 (46.7%)	8 (13.3.%)	10 (16.7%)
Gender N (%)					
female	31 (51.7%)	5 (8.3%)	18 (30.0%)	5 (8.3%)	3 (5.0%)
male	29 (48.3%)	9 (15.0%)	10 (16.7%)	3 (5.0%)	7 (11.7%)
Age at surgical intervention					
years mean	47.9	40.5	39.7	68.9	64.2

FITC-dextran flux across IEC-18 monolayers. After 24 hours preincubation with IFN-γ no significant difference in barrier permeability was detected (0.6±5.0%; p=0.487; Supplementary file Fig. 1). However, a significantly elevated permeability of the barrier was detected 48 hours after stimulation with IFN-γ (24.1±5.6%; p= 0.031) (Fig. 4), showing that a prolonged incubation with IFN-γ is needed to reduce epithelial monolayer integrity.
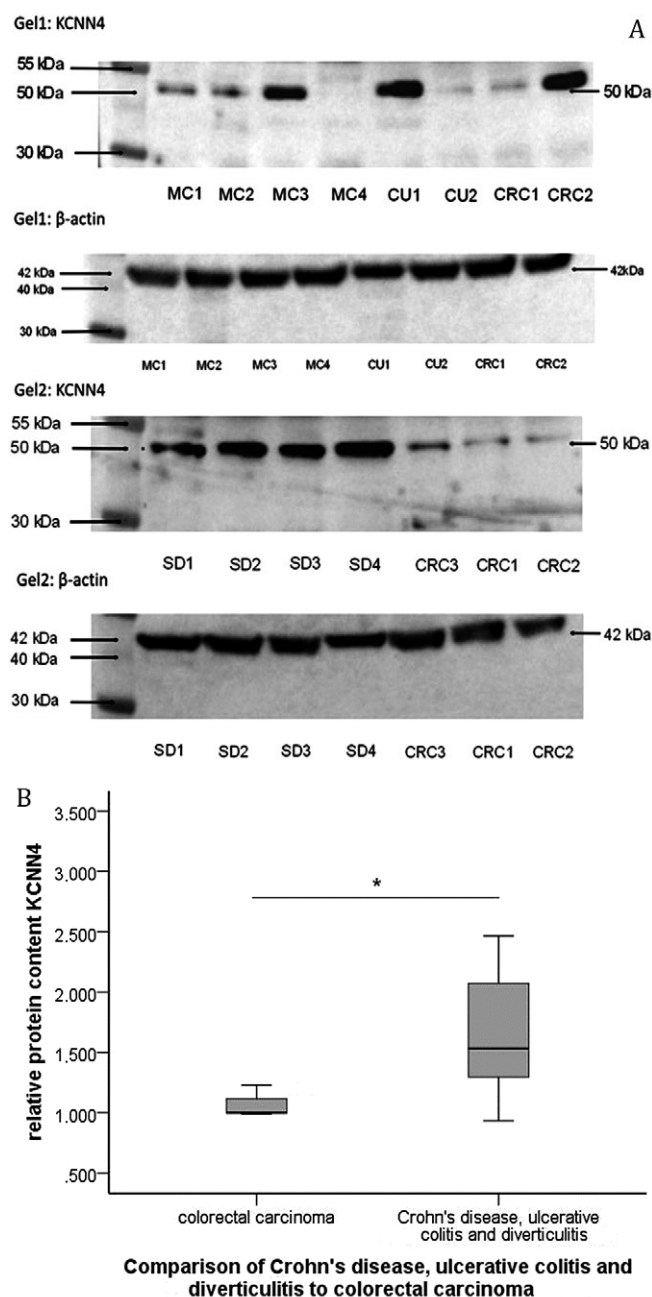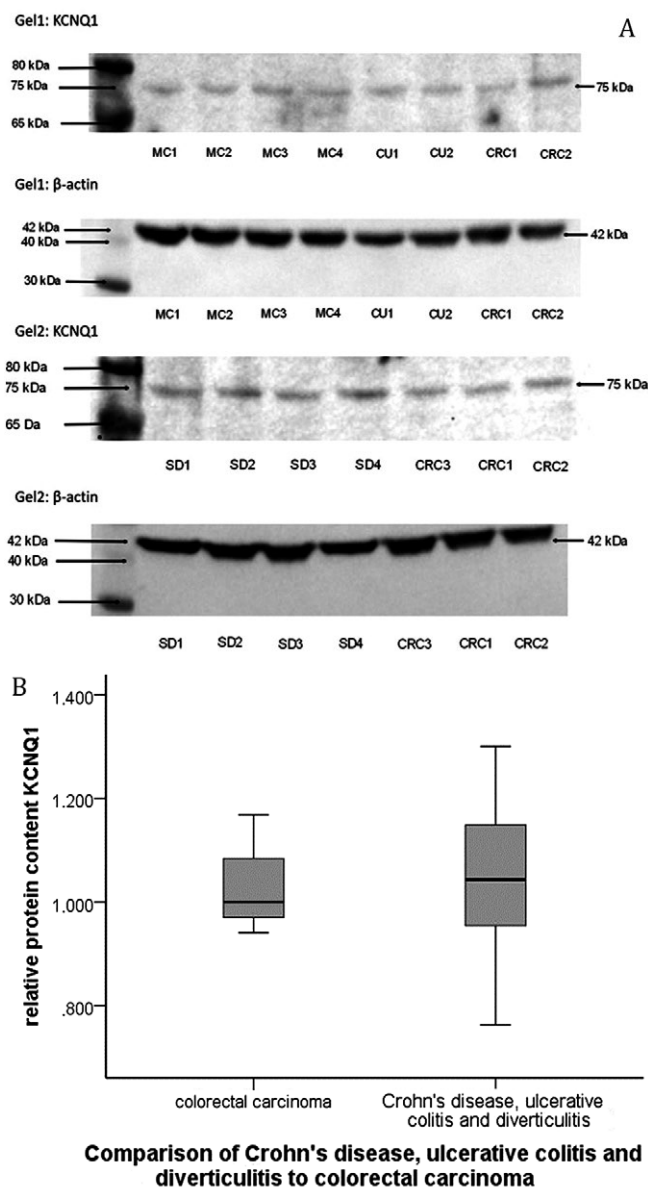

Fig. 2. KCNN4 protein levels are elevated in intestinal inflammation compared to uninflamed controls A) representative Western blot of whole cell lysates from primary IEC from patients with CD (n=4), UC (n=2), diverticulitis (n=4) and colorectal carcinoma (n=3), sequentially probed for KCNN4 or ß-actin as loading control. B) Densitometric analysis reveals significantly elevated KCNN4 levels in samples from patients with intestinal inflammation (CD, UC and diverticulitis together) compared to colorectal carcinoma as uninflamed controls. Data are expressed as -fold control. Asterisks denote statistically significant differences from controls (*, p ≤ 0.05).

Fig. 3. KCNQ1 protein levels as controls are similar in all groups A) representative Western blot of whole cell lysates from primary IEC from patients with CD (n=4), UC (n=2), diverticulitis (n=4) and colorectal carcinoma (n=3), sequentially probed for KCNQ1 or ß-actin as loading control. B) Densitometric analysis reveals similar KCNQ1 expression in samples from patients with intestinal inflammation (CD, UC and diverticulitis together) and in samples from patients with colorectal carcinoma as uninflamed controls. Data are expressed as -fold control. Asterisks denote statistically significant differences from controls (p ≤ 0.05).

We titrated TRAM-34 to define the optimal concentration for our experiments. For concentrations of 0.5 μM 1μM, 10μM and 25 μM no differences on membrane integrity were observed (Supplementary file Fig. 2). The KCNMA1 inhibitor iberiotoxin was used as a control [45]. In addition, two KCNN4 channel openers, namely 1-EBIO and SKA-31 were applied to evaluate KCNN4 as a potential therapeutic target.

The two KCNN4 inhibitors TRAM-34 (0.5±26.1%; p=0.51) and clotrimazole (11.7±32.5%; p=0.51) as well as the KCNMA1 inhibitor iberiotoxin (16.1±47.4%; p=0.51) did not influence the permeability compared to the respective control (Fig. 4).
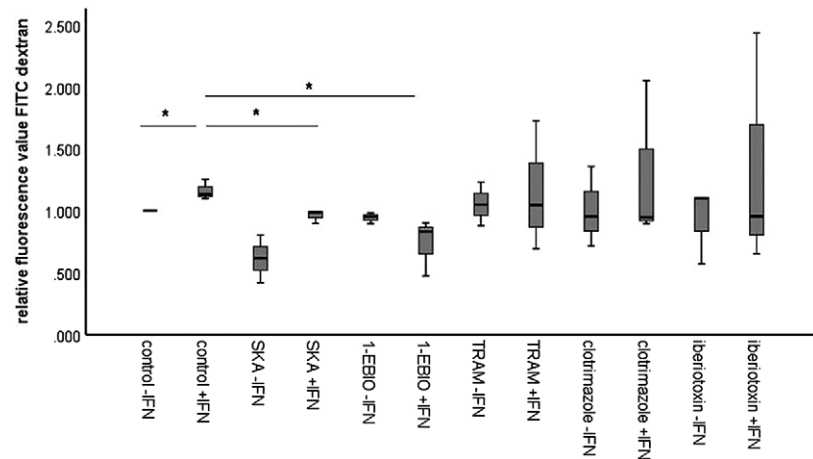
Fig. 4. FITC dextran flux in IEC18 cells after 48 hours of IFN-γ incubation is significantly elevated. The KCNN4 channel opener SKA-31 and the K+ channel opener 1-EBIO lead to a significantly reduced FITC dextran flux after 48 hours of IFN-γ incubation. Two KCNN4 inhibitors (TRAM-34 and clotrimazole) and a KCNMA1 inhibitor (iberiotoxin) have no significant influence on the FITC dextran flux after IFN-γ incubation (all performed in triplicate). Data are expressed as -fold control. Asterisks denote statistically significant differences from control (*, $p \leq 0.05$).

Of clinical relevance, the KCNN4 opener SKA-31 significantly reduced permeability of the cell monolayer ($-17.3 \pm 2.7\%$; $p=0.05$). An even stronger effect was observed after addition of 1-EBIO ($-36.7 \pm 11.5\%$; $p=0.05$) (Fig. 4). Thus, KCNN4 channel openers significantly improve gut epithelial monolayer integrity.

DISCUSSION

KCNN4 is the K+ channel which is expressed most prominently in the basolateral cell membrane of human IEC [46]. Recent data show that K+ channels are involved in the pathogenesis of chronic inflammation such as IBD or chronic airway disease [47]. The role of KCNN4 in IBD is controversially discussed since this K+ channel is expressed in different cell types such as Paneth cells, immune cells and IEC [48-50]. The impact of KCNN4 expression or activity was extensively analyzed in immune cells. T cell activation is altered by the expression level and activity of KCNN4 in T lymphocytes in pediatric patients with IBD [51]. The expression level of KCNN4 can be restored by anti-TNF therapy in these patients [52]. KCNN4 expression in T cells can be correlated with disease activity in UC [48]. Similarly, elevated T cell KCNN4 expression in UC correlates with inflammatory cytokines levels and disease activity [49]. In CD a gene variant of *KCNN4* has been described that is associated with ileal disease in the New Zealand and Australian population [30]. In a T-cell-induced mouse model of colitis it has been shown that a reduced KCNN4 activity in T-lymphocytes leads to a minor degree of inflammation [53].

We have demonstrated previously that inhibition of the Ca²⁺-activated K+ channel KCNN4 differentially regulates IEC migration in inflamed vs. non-inflamed conditions and that this is mediated by the PI3K signaling pathway [14]. Moreover, basolateral K+ channels function as the driving force for apical chloride secretion in IEC. It is therefore of special therapeutic interest if the expression of this channel is modulated in IEC from patients with IBD.

In this study, we analyzed the expression of the Ca²⁺-activated K+ channel KCNN4 in IEC isolated from surgical specimens of patients with IBD and controls. We evaluated the expression of *KCNN4* at mRNA and protein level in IEC from patients with CD, UC, diverticulitis and non-inflamed controls. In our experiments *KCNN4* mRNA was significantly increased in IEC from patients with chronic IBD, but not in patients with diverticulitis or colorectal carcinoma. This suggests an involvement of KCNN4 in development and course of chronic IBD. Moreover, not only *KCNN4* mRNA level, but also KCNN4 protein content was also augmented in IEC from patients with intestinal inflammation, whereas KCNQ1 protein content was not altered in all groups. This result emphasizes the importance of the Ca²⁺-activated K+ channel KCNN4 for IBD pathogenesis. As expression of KCNN4 is specifically elevated in patients with IBD, this could make KCNN4 an important and novel therapeutic target and/or diagnostic marker for IBD.

In IBD, epithelial barrier function is impaired and inflammatory cytokines such as IFN-γ are thought to mediate these alterations in the barrier function. IFN-γ is increased in CD in the intestinal epithelium [8]. We described earlier that IFN-γ causes a defective EGFR activation in IEC during inflammation. Alterations in downstream signaling pathways originating at EGFR play a pivotal role for ion transport responses as well as for intestinal restitution. We demonstrated that inhibition of KCNN4 leads to enhanced migration of IEC in non-inflamed tissues. However, in inflamed tissues, this effect was almost prevented [14]. Downregulation of tight junction proteins plays a central role in the destabilization of the barrier. It has been shown that K+ channels have an impact on tight junctions of epithelial cells [25, 26]. However, the exact role of KCNN4 in regulating epithelial barrier integrity needs to be elucidated. Since we had shown elevated KCNN4 levels in primary IEC of patients with IBD, we investigated whether

this K⁺ channel may display protective properties for epithelial barrier in IBD.

In this study we performed preincubation with IFN-γ in order to mimic inflammation in IEC-18-cells. This model was developed based on published data of other groups to investigate barrier function of cell monolayers following IFN-γ treatment [44, 45, 54]. As expected, we observed an increased permeability of the cell monolayer upon IFN-γ treatment. Remarkably and especially of clinical relevance, after addition of channel openers (e.g. 1-EBIO and SKA-31) epithelial barrier function was restored. Our data underline the important role of KCNN4 as a therapeutic target to stabilize epithelial barrier integrity in intestinal inflammation. In contrast, pharmacological inhibition of this K⁺ channel revealed no change of the permeability of the epithelial monolayer. A different K⁺ channel, the TWIK related K⁺ channel 1 (Trek 1) affects the epithelial barrier, but its expression is reduced by allergic responses [55].

CONCLUSION

Our data show that *KCNN4* mRNA expression is specifically increased in IBD but not in other intestinal inflammations e.g. diverticulitis. In clinical practice there is a diagnostic gap in some instances at the time of first diagnosis of IBD. In some cases, histologic results after the first endoscopy are unspecific. The lack of a reliable distinction between IBD and unspecific acute colitis (*e.g.* infectious colitis) often results in a delay of a specific therapy. In this study we show that *KCNN4* mRNA serves as a novel diagnostic marker for IBD which can be analyzed in biopsies obtained during endoscopy.

Therapeutic interference using KCNN4 channels openers might be protective in IBD. Opening of KCNN4 channels leads to stabilization of the epithelial barrier. Thus, K⁺ channels could be a promising novel target for IBD therapy due to the beneficial effects of KCNN4 openers on epithelial restitution.

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