ION CHANNELS, RECEPTORS AND TRANSPORTERS

Transient receptor potential canonical channel 1 impacts on mechanosignaling during cell migration

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Abstract Cell migration is crucial for many important physiological and pathophysiological processes ranging from embryogenesis to tumor metastasis. It requires the coordination of mechanical forces generated in different regions of the migrating cell. It has been proposed that stretch-activated, Ca^{2+}-permeable channels are involved in mechanosignaling during cell migration. To date, the molecular identity of these channels is only poorly defined. Here, we investigated the contribution of TRPC1 channels to mechanosignaling during cell migration. We used primary cultures of synovial fibroblasts from $TRPC1^{-/-}$ mice and the wild-type littermates or Madin–Darby canine kidney (MDCK-F) cells with increased or decreased TRPC1 expression. $TRPC1^{-/-}$ fibroblasts have the same migratory phenotype as siTRPC1 MDCK-F cells, with a largely increased projected cell area and impaired directionality. Measurements of the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) were combined with time-lapse video microscopic cell migration experiments. Cells were seeded on elastic silicone membranes. Uniaxial stretch elicits a graded elevation of the $[Ca^{2+}]_i$ in TRPC1-expressing cells. In contrast, $TRPC1^{-/-}$ fibroblasts or siTRPC1 MDCK-F cells do not react to 0.4 %, and the response to 4 % stretch is attenuated. Similarly, siTRPC1 MDCK-F cells do not alter their direction of migration upon mechanical stimulation, which contrasts the behavior of TRPC1-overexpressing cells which turn into the direction of stretch. Impaired mechanosignaling in siTRPC1 MDCK-F cells leads to accelerated lamellipodial protrusions. Finally, artificially decreasing membrane tension with the detergent deoxycholate impairs the migration of TRPC1-overexpressing cells, but not of siTRPC1 cells. Taken together, our findings indicate that TRPC1 channels are linked to mechanosignaling during cell migration.

Keywords Cell migration · TRPC1 · Calcium · Mechanosignaling

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Introduction

Cell migration plays an important role for many physiological and pathophysiological processes in health and disease. Prominent examples include embryogenesis, immune cells searching for invading pathogens, or tumor metastasis [28, 30]. On a cellular level, cell migration not only involves the transduction of mechanical forces onto the surrounding extracellular matrix but also requires the coordination of mechanical forces generated in different regions of the migrating cell. Different models have been proposed to account for the well-known impact of mechanical stimuli on cell migration. It has been proposed that stretch-activated, Ca^{2+}-permeable channels are involved in mechanosignaling during cell migration. In keratinocytes, the activation of mechanosensitive channels and the ensuing increase of the intracellular calcium concentration ($[Ca^{2+}]_i$) precede the retraction of their rear part [18]. Similarly, mechanosensitive Ca^{2+} channels have a distinctive impact on neurite outgrowth [15]. To date, the molecular identity of these channels, however, is largely unknown. It is discussed controversially whether transient receptor potential (TRP) channels could be involved. One of them, TRPC1, was reported to be directly activated by mechanical stimulation [22]. However, in a later study, this effect could not be reproduced [11], and TRPC1-deficient vascular smooth muscle cells showed the same pressure-induced cation

currents as wild-type cells [5]. On the other hand, TRPC1 channels are important components of mechanosensitive signaling during pressure overload-induced cardiac hypertrophy [29], and they contribute to the mechanosensation of cutaneous sensory neurons [9] and mechanical hyperalgesia [1]. The latter studies provide firm evidence that TRPC1 channels, while not necessarily mechanosensitive themselves, are intimately involved in processes depending on mechanosignaling.

We therefore set out to test whether TRPC1 channels could be part of the mechanosensitive signaling complex that is required for efficient cell migration. To this end, we combined time-lapse video microscopic migration experiments with ratiometric Ca^{2+} imaging using cells with altered TRPC1 expression (overexpression, siRNA-mediated knockdown and knockout) [5, 7, 8, 19].

Methods

Cell culture

Alkaline-transformed Madin–Darby canine kidney (MDCK-F) cells were cultivated in bicarbonate-buffered Minimal Essential Medium (MEM; pH 7.4) with Earle's salts (PAA Laboratories, Austria) containing 10 % fetal calf serum (Biochrom, Germany) in a 37 °C humidified atmosphere of 5 % CO_2. The experiments were carried out with two different cell lines: one cell line with a stably enhanced expression of the human N-terminally HA-tagged TRPC1 isoform and one with a stably reduced TRPC1 expression via short interfering RNA, as described [7, 8]. The cells are designated as TRPC1-HA or siTRPC1 cells.

Synovial fibroblasts from TRPC1$^{-/-}$ mice and wild-type littermates [5, 19] were isolated from deskinned hind paws. After dispase II digest (1.5 mg/ml; Roche Diagnostics GmbH, Germany), fibroblasts were cultured under standard conditions in DMEM supplemented with 10 % FBS (Invitrogen Corporation, USA) and antibiotics/antimycotics (100 U/ml penicillin G, 100 mg/ml streptomycin, 0.25 μg/ml amphotericin B; PAA Laboratories) as described previously [12]. Fibroblasts were used in passages 3–8. Experimental protocols were approved by the local Committee for Animal Care.

Migration experiments

The migration of MDCK-F cells was captured by means of time-lapse video microscopy, as described previously [7]. Cells were seeded in fibronectin-coated tissue culture flasks 1 day prior to the experiments. The culture vessels were placed into heated chambers (37 °C) mounted on inverted microscopes (Axiovert25, Carl Zeiss, Inc., Germany). Cell

migration was recorded using video cameras (models XC-ST70CE and XC-77CE, Hamamatsu/Sony, Japan) and PC-vision frame grabber boards (Hamamatsu, Herrsching, Germany). Acquisition of images was controlled by HiPic and WASABI software (Hamamatsu).

The deoxycholate experiments were performed in a paired fashion. We first monitored cell motility in normal growth medium (control) for 10 min. Then, sublytic amounts of deoxycholate (0.4 mM) were added and migration was recorded for another 10 min in 3-s intervals.

The migration of wild-type and TRPC1$^{-/-}$ fibroblasts was assessed in a "wound healing" assay. Cells were grown to confluency and then the monolayer was "wounded" with a pipette tip. After wounding the cell layer, the cell culture medium was replaced by one containing 5 ng/ml FGF-2 and wound closure was monitored for 16 h in 5-min intervals.

The outlines of the cells were marked at each time step throughout the entire image stack applying the AMIRA software (Visage Imaging GmbH). The cell contours then served as the basis for further analysis. Parameters such as cell area (in square micrometers), migratory speed (in micrometers per minute), and translocation (in micrometers) were analyzed using self-made JAVA programs and the NIH ImageJ software (http://rsb.info.nih.gov/ij/). Migration was determined as the movement of the cell center as a function of time. Mean translocation represents the distance covered during the course of the experiments. The directionality of movement was estimated by plotting the distance of the cells traveled in the direction perpendicular to the wound (x direction) as a function of time (x-translocation).

Mechanical stimulation of migrating cells

Two days prior to the experiment, the cells were seeded on fibronectin-coated (1 μg/cm^2) stretchable silicone chambers ("Cell Warp" from AdvancedLAB, Austria). The cells were pre-incubated with Ringer's solution. The experiments were carried out at 37 °C and the cells continuously superfused with Ringer's solution. Cell migration was recorded by bright-field video microscopy in 60-s intervals. CoolSnap camera and data acquisition were controlled by Metafluor Software (Visitron Systems). After a 25-min control period, the cells were subjected to a sustained 0.4 or 4 % uniaxial stretch which was applied by a manually controlled micromanipulator (Cell Warp). The silicone membrane had acquired its new length after ~2 or ~5 s, respectively.

Cell speed and directionality were analyzed by tracking the x/y movement of the cells' center as a function of time and in relation to the direction of the stretch stimulus which was aligned along the y-axis of a virtual coordinate system. Directional movement in response to mechanical stimulation was revealed by analyzing the mean velocities in the x and y directions separately. The x- and y-axes are vertical

and parallel to the direction of the mechanical stimulus, respectively.

Intracellular Ca^{2+} measurements during mechanical stimulation

Two days prior to the experiment, the cells were seeded on fibronectin-coated (1 µg/cm^2) stretchable silicone chambers ("Cell Warp" from AdvancedLAB). For dye loading, the cells were pre-incubated with Ringer's solution containing fura2-AM (3 µM; Calbiochem) at RT for 25 min. The experiments were carried out at 37 °C and the cells continuously superfused with Ringer's solution.

$[Ca^{2+}]_i$ in single cells was measured ratiometrically before and after mechanical stimulation (0.4 and 4 %) in a paired fashion; see "Mechanical stimulation of migrating cells." Excitation wavelengths alternated between 340 and 380 nm. The emitted fluorescence was monitored at 500 nm. The mean cellular fluorescence intensities were corrected by background subtraction and measured in 20-s intervals. At 0.5 min before and 2 min after mechanical stimulation, acquisition intervals were reduced to 3 s. $[Ca^{2+}]_i$ was calculated as described before [6]. Briefly, Ca^{2+} measurements were calibrated at the end of each experiment. Maximal and minimal ratios were determined separately for each cell following the application of Ringer's solutions containing ionomycin (1 µM) and either 5 mM EGTA or 5 mM Ca^{2+}. For each cell clone, at least 32 cells were analyzed. We show changes of the mean peak values of $[Ca^{2+}]_i$.

Statistical analysis

Data are presented as the mean ± SEM. Statistical significance was tested with paired or unpaired Student's t test ($p < 0.05$) as appropriate.

Results

Intracellular Ca^{2+} concentration changes in response to mechanical stimulation

Our previous studies indicated that TRPC1 channels play an important role in the directionality of MDCK-F cell migration by regulating the $[Ca^{2+}]_i$ [7, 8]. In a first set of experiments, we tested whether a diminished mechanical responsiveness of siTRPC1 cells could at least partially account for the previously observed differences in intracellular Ca^{2+} signaling. The results of these experiments are presented in Fig. 1. MDCK-F cells overexpressing TRPC1 channels react with a graded response to mechanical stimulation, as illustrated by one representative experiment shown in Fig. 1a. Following some irregular spontaneous oscillations, 0.4 % stretch elicited a sharp peak of the $[Ca^{2+}]_i$. Thereafter, the $[Ca^{2+}]_i$ returns to baseline. A 4 % stretch augmented this response, and the $[Ca^{2+}]_i$ remains at an elevated level. In contrast, siTRPC1 cells hardly react to mechanical stimulation. Figure 1b exemplifies such an experiment. The $[Ca^{2+}]_i$ of siTRPC1 cells remains unaffected by 0.4 % stretch, and the rise of the $[Ca^{2+}]_i$ after 4 % stretch is largely attenuated. Figure 1c, d summarizes the experiments by showing the
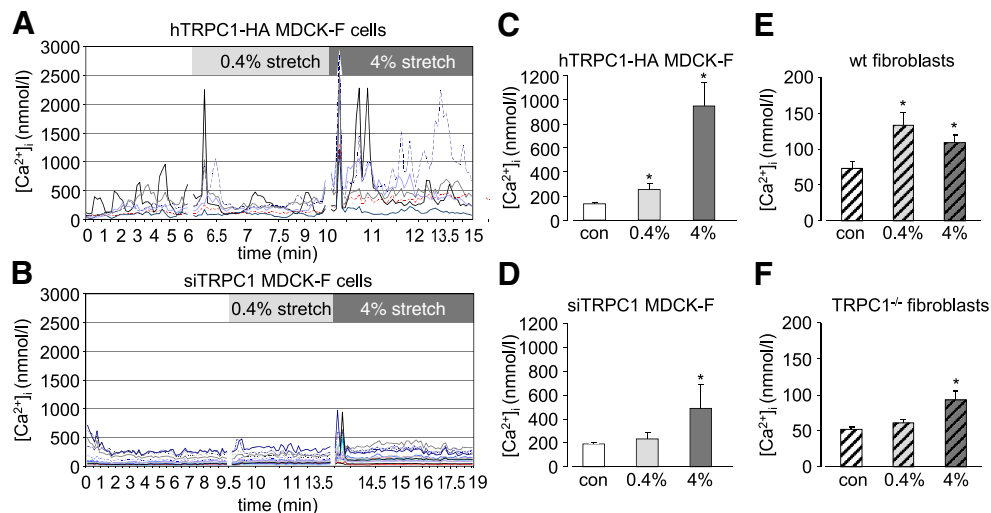

Fig. 1 TRPC1 expression is linked to stretch-induced Ca^{2+} signaling. a, b Representative traces of the $[Ca^{2+}]_i$ of MDCK-F cells under control conditions and after stretches of 0.4 and of 4 %. In hTRPC1-HA-overexpressing MDCK-F cells (a), 0.4–4 % stretch induces graded increases of $[Ca^{2+}]_I$, which are largely attenuated in siTRPC1 MDCK-F cells (b). c, d Mean peak increase of the $[Ca^{2+}]_i$ following mechanical stimulation in hTRPC1-HA-overexpressing and in siTRPC1 MDCK-F cells, respectively. e, d Summary of analogous experiments performed with fibroblasts isolated from wild-type (e) and TRPC1$^{-/-}$ mice (f). Mechanically induced transients of the $[Ca^{2+}]_i$ are blunted in fibroblasts from TRPC1$^{-/-}$ mice. Please note the different scale of the ordinates in c, d and e, f

mean peak increase of the $[Ca^{2+}]_i$ after mechanical stimulation of hTRPC1-HA-overexpressing cells (0.4 % stretch, $n=53$; 4 % stretch, $n=32$) or siTRPC1 cells (0.4 % stretch, $n=53$; 4 % stretch, $n=54$).

To further confirm the contribution of TRPC1 channels to the mechanically induced rise of the $[Ca^{2+}]_i$ and to rule out the responsiveness of TRPC1-overexpressing cells to mechanical stimulation being an artifact of the overexpression, we performed similar experiments with synovial fibroblasts from wild-type and TRPC1$^{-/-}$ mice (Fig. 1e, f). Fibroblasts behave in a very similar way. Stretching them by 0.4 % fails to elicit a rise of the $[Ca^{2+}]_i$ in TRPC1$^{-/-}$ cells ($n=$ 61), while it is almost doubled in wild-type cells ($n=$ 76). Only a 4 % stretch induces a change of the $[Ca^{2+}]_i$ in both cell strains ($n \geq 62$). Collectively, these experiments indicate that the downregulation or knockout of TRPC1 channels in MDCK-F cells or synovial fibroblasts, respectively, leads to a decreased mechanosensitivity of both cell types.

Migration of TRPC1$^{-/-}$ fibroblasts

Synovial fibroblasts from TRPC1$^{-/-}$ mice not only behave similar to siTRPC1 MDCK-F cells with respect to mechanically induced intracellular Ca^{2+} signalling but also have a similar defect in cell migration, as revealed by the time-lapse video microscopic "wound healing" assays displayed in Fig. 2. The restitution of the cell layer occurs much more slowly with TRPC1$^{-/-}$ fibroblasts than with wild-type cells (Fig. 2a). Consequently, the paths of individual cells are shorter for TRPC1$^{-/-}$ than for wild-type fibroblasts (Fig. 2b), which is reflected by the overall reduction of the translocation from 100.7 ± 6.0 μm (wild-type, $n=80$) to 54.0 ± 4.6 μm (TRPC1$^{-/-}$, $n=54$), which corresponds to a decrease of 46.4 %. A closer analysis of the migratory behavior of the synovial fibroblasts is shown in Fig. 2c, d. The speed of TRPC1$^{-/-}$ fibroblasts (0.19 ± 0.01 μm/min) is ~27 % lower than that of the wild-type cells (0.26 ± 0.01 μm/min). In addition, TRPC1$^{-/-}$ fibroblasts have a strong defect of their directionality so that their translocation into the wounded area ("x-

Fig. 2 TRPC1 knockout leads to the impaired migration of fibroblasts. **a** Images taken from time-lapse sequences of migrating wild-type and TRPC1$^{-/-}$ fibroblasts. Images are depicted in 3-h intervals. The *dashed lines* represent the advancement of the wound edge which is more rapid in cultures of wild-type fibroblasts. **b** Trajectories of individual fibroblasts normalized to a common starting point. **c** Average speed of individual fibroblasts during wound closure. **d** The directionality of migrating fibroblasts is assessed by plotting the translocation into the wound (x direction, see also **a**). The directional migration of TRPC1$^{-/-}$ fibroblasts has almost halved
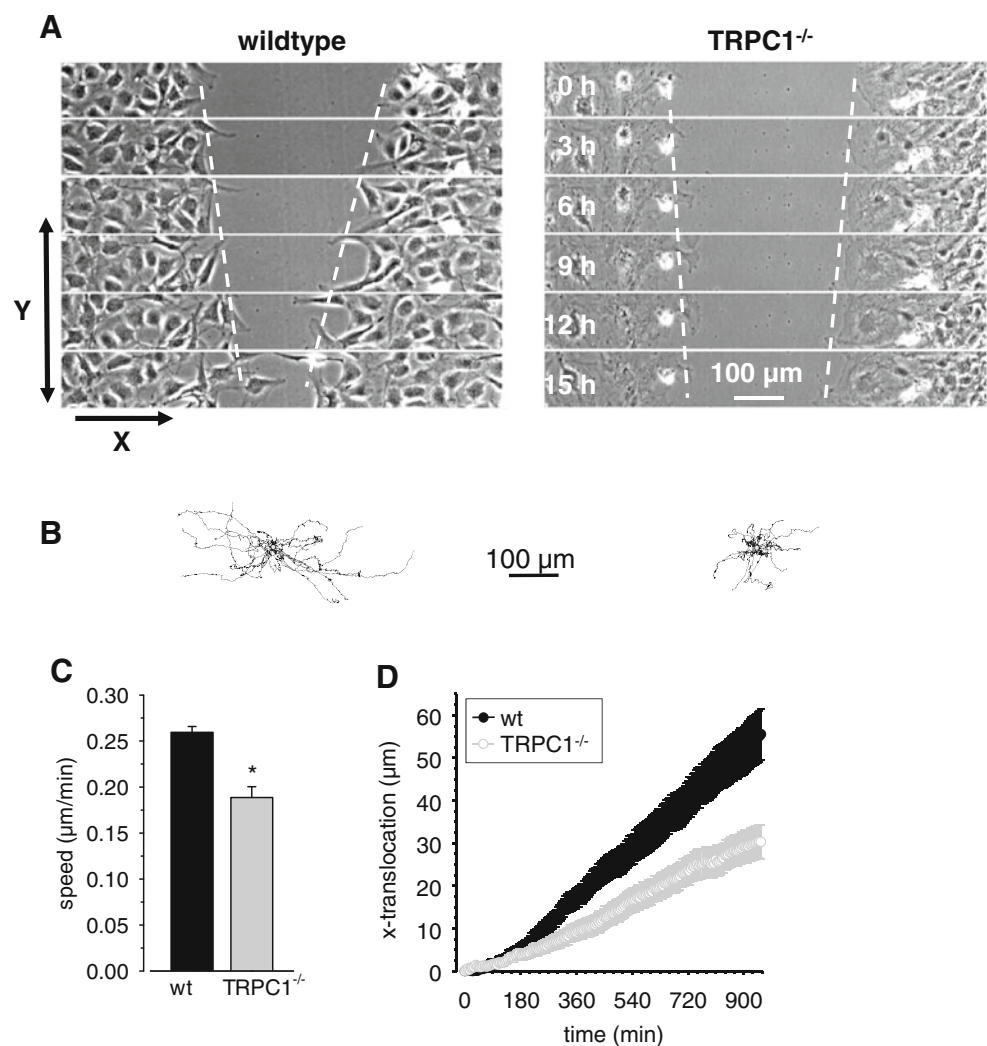
translocation" in Fig. 2d) is reduced by 45.4 % (55.5±6.0 versus 33.9±3.9 μm). Finally, the projected cell area of TRPC1$^{-/-}$ synovial fibroblasts is approximately twice as large as that of wild-type fibroblasts (12,513±1055 versus 5,653± 446 μm^2). Thus, TRPC1 knockout in fibroblasts leads to the same migratory and morphological phenotype as TRPC1 downregulation in MDCK-F cells, namely, primarily to a strongly impaired ability for directional migration and to a doubling of the cell area [7].

Mechanical stimulation of migrating MDCK-F cells

In the next set of experiments, we investigated the involvement of TRPC1 channels in mechanosensing during cell migration more directly. Cells were seeded on an elastic silicone membrane and the migratory behavior was quantified in paired experiments before and after stretching the membrane uniaxially by 0.4 %. The results of these experiments are summarized in Fig. 3 (TRPC1-HA cells, n=25) and Fig. 4 (siTRPC1 cells, n=25). Figure 3a depicts the trajectories of individual TRPC1-overexpressing cells observed before (left panel) and after the application of stretch which is applied in the y direction (right panel). The absolute speed of both cell types is not changed by the stretch stimulus (TRPC1-HA cells, 1.06±0.07 and 1.15±0.08 μm/min; siTRPC1 cells, 0.93±

0.06 and 0.98±0.07 μm/min before and after the stretch, respectively). However, TRPC1-HA MDCK-F cells adopt a preferred direction of migration in parallel to the stretch following mechanical stimulation. We quantified this response by determining the cells' speeds in the x (v_x) and y directions (v_y), i.e., perpendicular and parallel to the stretch stimulus, respectively. As expected, the speeds in v_y and v_x are virtually identical under control conditions (0.79±0.02 versus 0.67± 0.02 μm/min, n=25). However, following the mechanical stretch, v_y is almost twice as high as v_x (0.91±0.03 versus 0.56±0.01 μm/min). In contrast, siTRPC1 cells fail to respond to the mechanical stimulation, as shown in Fig. 4a, b. The trajectories (Fig. 4a), as well as the time course of v_y and v_x (Fig. 4b), show that the stretch in the direction of the y-axis does not elicit a change in the direction of migration. v_y and v_x do not change in response to the stretch stimulus: v_y=0.75± 0.02 μm/min and v_x=0.68±0.02 μm/min under control conditions versus 0.75±0.02 and 0.65±0.02 μm/min following stretch. These findings are consistent with our previous results that migration of siTRPC1 cells is hardly affected by the blocker of stretch-activated channels, GsMTx-4 [7].

Finally, we performed control experiments to rule out that stretching the silicone membrane and the ensuing alignment of the fibronectin coating itself is responsible for the altered direction of the migration of TRPC1-HA-overexpressing cells. We therefore coated the unstretched silicone membrane with fibronectin and applied the stretch before seeding
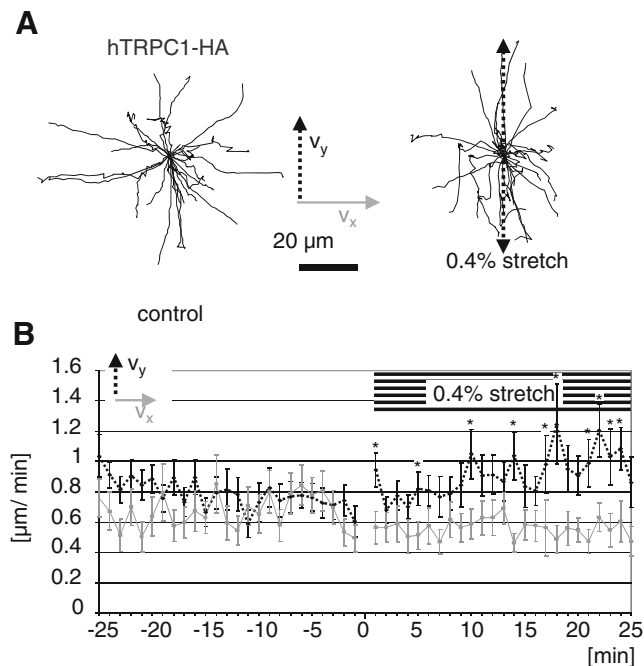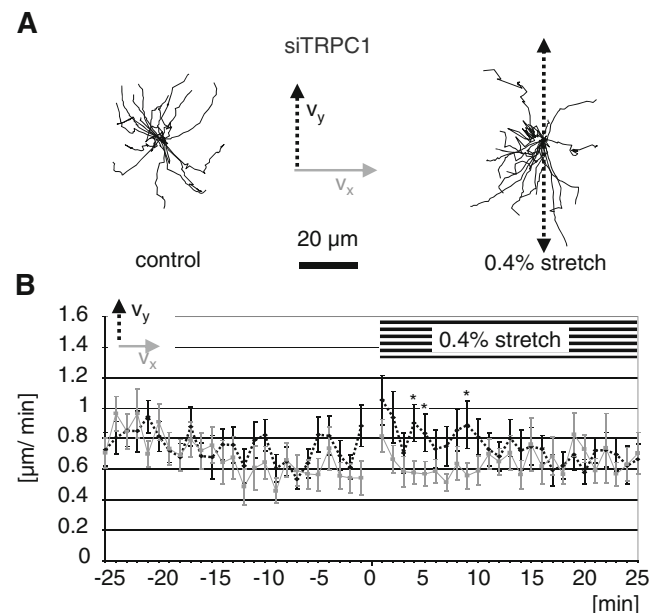

Fig. 3 Matrix stretching steers TRPC1-HA-overexpressing MDCK-F cells. TRPC1-HA-overexpressing MDCK-F cells were seeded on flexible silicone membranes. **a** Random trajectories of cells migrating under control conditions (*left*) and trajectories of the same cells after 0.4 % uniaxial stretch in the y direction. **b** Time course of the mean velocities in the x and y direction (v_x and v_y) before and after the stretch stimulus. v_y is increased following mechanical stimulation, while v_x is decreased

Fig. 4 Silencing of TRPC1 impairs mechanical guidance. **a** Trajectories of siTRPC1 MDCK-F cells under control conditions (*left panel*) and of the same cells after 0.4 % uniaxial stretch into the y direction (*right panel*). **b** Mean velocities in the x and y directions (v_x and v_y) before and after the stretch stimulus plotted as a function of time. The mechanical stimulus has hardly any effect on the directionality of siTRPC1 MDCK-F cell migration
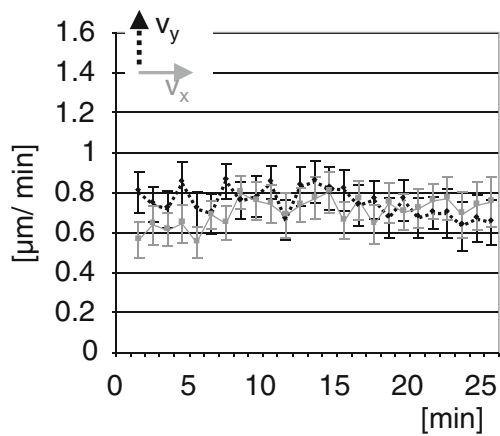
Fig. 5 Pre-stretched matrices do not impact on the directionality of cell migration. After coating with fibronectin, the silicone membranes were stretched before seeding the cells. The directionality of cell migration was as random as on an unstretched silicone membrane

MDCK-F cells. In Fig. 5, we plotted the time course of the v_y and v_x of MDCK-F cells ($n=25$) migrating on pre-stretched membranes. There is no preference in the direction of migration so that we can rule out that a stretch-dependent alignment of the fibronectin matrix underlies the response to mechanical stimulation. Taken together, the migration experiments lend further support to the notion that TRPC1 channels participate in coordinating the response of MDCK-F cells to mechanical stimulation.

Membrane tension affects cell migration of MDCK-F cells depending on TRPC1 expression

The observations described above are consistent with the notion that TRPC1 ablation causes an inability of the cells to sense membrane tension. An increase in membrane tension itself has been shown to slow down lamellipodial protrusion [10]. We therefore tested whether the impaired ability for mechanosensation of cells with reduced TRPC1 expression leads to an accelerated rate of lamellipodial protrusion. To this end, we monitored migration at high time resolution

($\Delta t=3$ s between two consecutive images). Under these experimental conditions, the calculated speed of migration is largely determined by lamellipodial dynamics [4]. Indeed, siTRPC1 MDCK-F cells are faster than TRPC1-HA MDCK-F cells: 3.46 ± 0.26 μm/min ($n=25$) versus 2.70 ± 0.15 μm/min ($n=36$; Fig. 6a).

In migrating cells, mechanosensitive channels are required for triggering the retraction of the rear part and, thereby, for the translocation of the entire cell [18, 20]. Here, we tested the effect of artificially decreasing membrane tension with the detergent deoxycholate. We measured translocation in paired experiments before and after the addition of 0.4 mM deoxycholate (Fig. 6b). In hTRPC1-HA cells, the translocation (within 10 min) is reduced from 8.8 ± 0.9 μm under control conditions to 4.5 ± 0.5 μm in the presence of the detergent ($n=25$). In contrast, the translocation of siTRPC1 cells is not affected by deoxycholate (control, 6.1 ± 1.0 μm; deoxycholate, 5.2 ± 0.6 μm; $n=25$). These experiments lend further support to the idea that TRPC1 channels are involved in mechanosensing during MDCK-F cell migration. However, we are aware that we cannot dismiss the possibility that deoxycholate affects migration by stimulating store-operated calcium [2] entry, which in part relies on TRPC1 expression in MDCK-F cells [21].

Discussion

The key observation of our study is that TRPC1 channels are involved in adjusting cell migration to external mechanical stimuli. The downregulation or knockout of TRPC1 channels in MDCK-F cells or synovial fibroblasts decreases their sensitivity to mechanical stimulation. Notably, both cell types have lost the ability to respond to a mild mechanical stimulation of a 0.4 % increase in length. No elevation of the $[Ca^{2+}]_i$ and no change in the direction of migration is induced in TRPC1-ablated cells, which is in contrast to TRPC1-(over-)expressing cells. However, a stronger mechanical stimulation still elicits a Ca^{2+} response. This
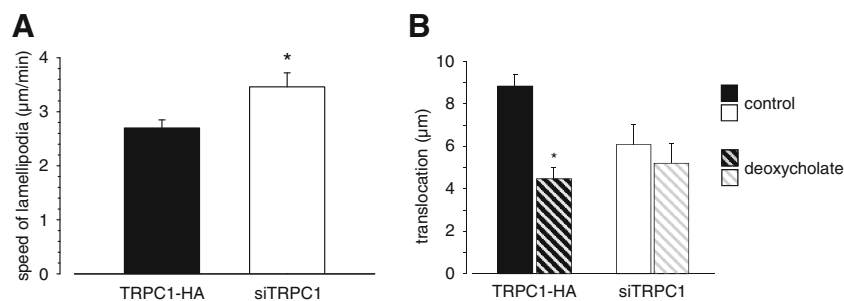

Fig. 6 Membrane tension impacts on cell migration. **a** The speed by which lamellipodia protrude is higher in siTRPC1 MDCK-F cells. **b** Following a 10-min control period, deoxycholate was added (0.4 mM) to migrating MDCK-F cells to reduce membrane tension. The

translocation of MDCK-F cells overexpressing hTRPC1-HA is decreased after the application of the detergent. Deoxycholate treatment has no effect on the translocation of siTRPC1 MDCK-F cells

differential response is reminiscent of observations made in sensory neurons. TRPC1 knockout only affects the response to light touch, but not to stronger nociceptive stimuli [9]. Stretching cells by 0.4 % is of a magnitude that MDCK-F cells encounter physiologically during the protrusion of their lamellipodia. MDCK-F cells have an average length of approximately 50 µm [27]. The length is increased by ~0.25 µm when the cells are stretched by 0.4 %. Stretching itself occurs within ~2 s. Thus, the increase in cell length occurs with a rate of ~6 µm/min. While faster than the average cell movement, such a speed can be reached locally and temporarily by protruding lamellipodia.

The fact that stronger mechanical stimulation still elicits increases of the $[Ca^{2+}]_i$ in TRPC1$^{-/-}$ fibroblasts indicates that these channels cannot be the only Ca^{2+}-permeable channels involved in mechanosensation. Other ubiquitously expressed TRP channels could be involved. Thus, TRPV4 channels that are expressed in synovial fibroblasts [14] could come into play. In endothelial cells, a mechanical load imposed via β_1 integrins induced an ultra-rapid Ca^{2+} influx via TRPV4 channels [23]. Similarly, TRPM7 channels could take over the role of TRPC1 channels [26, 32, 34]. The mechanosensitivity of TRPC1 channels is discussed controversially [11, 22], and our study does not allow ascribing the mechanosensitivity to the channel protein itself. Presently, we cannot distinguish whether TRPC1 channels are the primary sensor or rather a downstream signaling module transducing mechanical signals. The latter could involve mechanosensitive G-protein-coupled receptors triggering the activation of $G_{q/11}$ proteins and PLC. The resulting generation of diacylglycerol would then stimulate TRPC channels [24, 31].

The important question is why the mechanical stretch induces a change in the direction of movement only in TRPC1-expressing and not in TRPC1-silenced MDCK-F cells. An orientation of migrating cells in the direction of a mechanical stimulus also occurs in other cell types such as endothelial cells, trophoblasts, or vascular smooth muscle cells [3, 16, 17]. In vascular smooth muscle cells, this behavior was explained on the basis of differential Rac activation. Rac is inhibited at the sides where the tangential tension is effective, while it is activated at the ends of uniaxially stretched cells [17]. Similarly, membrane stretch causes Rac inhibition in neutrophils [13]. In analogy to the studies on the mechanical regulation of Rac activity, we would like to propose that TRPC1 channels could play a similar role as mechanosensitive regulators of protrusive activity. In such a scenario, the intracellular Ca^{2+} transients induced by mildly increasing tangential tension lead, together with Rac inhibition, to the retraction of the lamellipodia or to the suppression of their formation at the sides (vertically to the direction of stretch) of TRPC1-expressing cells. Conversely, lamellipodia formed in the direction of stretch

are favored. In migrating endothelial cells or *Dictyostelium discoideum*, a close temporal correlation was revealed between local elevations of the $[Ca^{2+}]_i$ and the retraction of lamellipodia [33] or of the rear end, respectively [20].

The lack of mechanosensitive Ca^{2+} signaling could also provide an explanation for the striking morphological and migratory phenotype of siTRPC1 MDCK-F cells [7] and TRPC1$^{-/-}$ synovial fibroblasts. Protrusion of the lamellipodium and cell spreading lead to an increase of the plasma membrane tension [10], which could eventually cause the activation of mechanosensitive Ca^{2+} channels [18]. If mechanically triggered Ca^{2+} influx occurred at the cell front, as previously suggested [25, 34], it could balance uncontrolled lamellipodial outgrowth [33]. Our present results indicate that TRPC1 channels are involved in mechanosensation, Thus, the largely increased cell area and the augmented protrusive activity of siTRPC1 or TRPC1$^{-/-}$ cells could be a consequence of a perturbed local Ca^{2+} homeostasis at the front or at the sides of the cells.

Taken together, our study shows that TRPC1 channels are central elements of mechanosignaling in migrating cells. Future studies will have to address the question whether this is due to a functional coupling with mechanically activated G-protein-coupled receptors or not.

Conflict of interest The authors declare that they have no conflict of interest.

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