Identification of SK3 channel as a new mediator of breast cancer cell migration

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Abstract

Potassium channels have been involved in epithelial tumorigenesis but the role of small-conductance Ca^{2+}-activated K^+ channels is unknown. We report here that small-conductance Ca^{2+}-activated K^+ channels are expressed in a highly metastasizing mammary cancer cell line, MDA-MB-435s. Patch-clamp recordings showed typical small-conductance Ca^{2+}-activated K^+ channel–mediated currents sensitive to apamin, 4-aminopyridine, and tetraethylammonium. Moreover, the cells displayed a high intracellular calcium concentration, which was decreased after 24 hours of apamin treatment. By regulating membrane potential and intracellular calcium concentration, these channels were involved in MDA-MB-435s cell migration, but not in proliferation. Only SK3 protein expression was observed in these cells in contrast to SK2, which was expressed both in cancer and noncancer cell lines. Whereas small interfering RNA directed against SK3 almost totally abolished MDA-MB-435s cell migration, transient expression of SK3 increased migration of the SK3-deficient cell lines, MCF-7 and 184A1. SK3 channel was solely expressed in tumor breast biopsies and not in nontumor breast tissues. Thus, SK3 protein channel seems to be a new mediator of breast cancer cell migration and represents a potential target for a new class of anticancer agents. [Mol Cancer Ther 2006;5(11):2946–53]

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Introduction

Numerous studies have shown that potassium (K^+) channels interfere with pathways controlling the balance between cell growth and cell death (1, 2). In contrast, the role of K^+ channels in tumor cell dissemination and metastasis has been less intensively investigated. Migration is one of the crucial steps in the metastatic cascade, which is, in part, responsible for death in cancer. Some of the processes involved in cell migration, including the contraction of the actomyosin network and the disassembly of cell–substratum adhesions, can be regulated by intracellular Ca^{2+}. For example, high Ca^{2+} concentration promotes the retraction of the rear part of the migrating cell through the activation of Ca^{2+}-dependent enzymes. Indeed, a Ca^{2+}-calmoduline–dependent phosphatase and a Ca^{2+}-dependent focal adhesion kinase were found to be involved in the cell edge retraction (3, 4). However, how the increase of intracellular Ca^{2+} is generated is still not known in most cases. In nonexcitable cells, such as epithelial cells, a raise in intracellular Ca^{2+} could be promoted by K^+ channels, which, by hyperpolarizing the membrane, lead to an increase of the Ca^{2+} electrochemical driving force and of Ca^{2+} influx.

According to the ability of Ca^{2+}-activated potassium (KCa) channels to control intracellular Ca^{2+} concentration, we hypothesized that these channels are involved in cell migration. KCa channels, a family of K^+ channels, comprise many channels that differ in their primary amino acid sequences and exhibit different single-channel conductances and pharmacologic profiles (5). Therefore, KCa channels can be divided into three subfamilies: large- or big-conductance KCa, intermediate-conductance KCa, and small-conductance KCa. Small-conductance KCa and intermediate-conductance KCa channels are voltage insensitive and are activated by low concentrations of intracellular Ca^{2+} in contrast to big-conductance KCa channel, which is activated by both voltage and intracellular CA^{2+} (5). Neither small-conductance KCa nor intermediate-conductance KCa channels bind Ca^{2+} directly but rather detect Ca^{2+} through its association with calmodulin, which is a part of the channel complex (6). Functional K_{Ca} channels result mostly in homomeric or heteromeric assemblies of α subunits. There are three isoforms of small-conductance KCa α subunits, named SK1, SK2, and SK3, which associate to form homotetramers or heterotetramers (7, 8). SK1, SK2, and SK3 proteins are principally expressed in central neurons where small-conductance KCa channels have a fundamental role in regulating neuronal excitability (9). Indeed, they hyperpolarize the plasma membrane and they contribute to the long-lasting posthyperpolarization that follows an action potential (9). SK2 and SK3, in contrast to SK1, are not restricted to neuronal tissues (10). If the role of SK2 channel is not shown outside central neurons, activation of SK3 channel was found to regulate smooth

muscle tone by inducing hyperpolarization (11–13). Among KCa channels, intermediate-conductance KCa (composed of SK4 protein that is strictly expressed outside the central nervous system) seems to be involved in the migration process by facilitating the retraction of the rear part of migrating cells through induction of local cell shrinkage (14).

In this report, we present evidence for the involvement of the SK3 channel in the migrating capacity of one of the most metastasizing human mammary cancer cell lines, MDA-MB-435s (15, 16). As SK3 expression is restricted to tumor breast tissues, we propose that SK3 channels represent a new phenotypic marker of mammary epithelial cell transformation mediating cell migration.

Materials and Methods

Cell Culture

The human mammary cancer cell lines MDA-MB-435s, MDA-MB-231, MCF-7, T47D, and SKBR3 were grown in DMEM containing 5% fetal bovine serum as already described (17). The immortalized normal mammary epithelial cell lines MCF-10A and 184A1 were cultured in DMEM/Ham's F-12, 1:1 mix containing 5% horse serum (Invitrogen Life Technologies, Cergy Pontoise, France), insulin (10 μg/mL), epidermal growth factor (20 ng/mL), hydrocortisone (0.5 μg/mL), and, for MCF-10A and 184A1, 100 ng/mL cholera toxin and 1 ng/mL cholera toxin plus 5 μg/mL transferrin, respectively.

High K^+ medium was custom-made from K^+-, Na^+-, and Ca^{2+}-free DMEM-based medium (Cambrex BioScience, Verviers, Belgium) and supplemented at time of use at 60 mmol/L KCl, 84 mmol/L NaCl, and 2 mmol/L $CaCl_2$.

All cell lines were obtained from the American Type Culture Collection (LGC Promochem, Molsheim, France).

Breast Tissue Samples

Tissue samples were provided from patients treated by surgery in the University Hospital of Tours in 1991. Tumor and nontumor tissue samples were selected by a pathologist from fresh specimens and directly frozen in liquid nitrogen until analysis. After thawing of tumors, imprints were done, stained with May-Grunwald-Giemsa, and observed by the pathologist to verify for the presence of malignant cells. Control tissue samples were chosen among patients with aneuploid tumors and analyzed by flow cytometry to verify the absence of aneuploid tumor cells.

Cell Proliferation and Cell Migration *In vitro*

Cell proliferation was determined using the tetrazolium salt reduction method, as described (17). Cells were seeded on 24-well plates and grown for 48 hours. Drugs were then added for 24 hours at concentrations that had no effect on cell proliferation. Cell migration was analyzed in 24-well plates receiving 8-μm pore size polyethylene terephtalate membrane cell culture inserts (Becton Dickinson, Pont de Clais, France), as described (17).

Electrophysiology and Intracellular Ca^{2+} Measurements

Whole-cell potassium currents and intracellular Ca^{2+} measurements were recorded as described (17). Signals were captured using 1322-A Digidata converter (Axon Instruments, Union City, CA) and pClamp 8.1 software (Axon Instruments). The analyses were done using Clampfit 8.1 and Origin 7.0 softwares (Microcal Software, Northampton, MA).

Reverse Transcription-PCR and Western Blot

Reverse transcription-PCR experiments were done according to standard protocols. The following primers were used: SK-2 5′-primer GACTTGGCAAAGACCCAGAA and 3′-primer CCGCTCAGCATTGTAAGTGA (231 pb) and SK3 5′-primer TGGACACTCAGCTCACCAAG and 3′-primer GTTCCATCTTGACGCTCCTC (174 pb). The ribosomal gene RNA *S14* was chosen as the housekeeping gene using the following PCR primers: 5′-primer GGCAGACCGAGATGAATCCTCA-3′ and 3′-primer CAGGTCCAGGGGTCTTGGTCC-3′.

For Western blot experiments, proteins were electrotransferred onto polyvinylidene fluoride membranes that were incubated with antibodies directed against SK2 and SK3 proteins (1:1,000) followed by incubation with a horseradish peroxidase–conjugated anti-rabbit IgG (1:5,000; Tebu-Bio, Le Perray-en-Yveliae, France). Anti-SK2 directed against amino acids 542 to 559 and anti-SK3 directed against amino acids 2 to 21 (Sigma-Aldrich, St Quentin, France) were used for Western blot and immunocytochemical experiments. Anti-actin directed against amino acids 20 to 33 (Sigma-Aldrich) was used for Western blot loading control experiments.

Synthesis and Transfection of Small Interfering RNA Directed against SK3

Two SK3-specific small interfering RNA (siRNA) were designed: first set, hSK3-ex1-sense 5′-GAAAGCGACUGAGUGACUAdTdT-3′ and hSK3-ex1-antisense 5′-UAGUCACUCAGUCGCUUCdTdT-3′, located in exon 1; second set, hSK3-ex3-sense 5′-CCAUUCCUGGCGAGUACAAdTdT-3′ and hSK3-ex3-antisense 5′-UUGUACUCGCCAGGAAUGGdTdT-3′, located in exon 3. The negative control siRNA (scramble) used had the following sequence 5′-AUAACUGUAUCGAAUGUUAUGAGCC-3′. Transfections were done as previously described (18).

Transient Transfection of SK3 Protein Channel

The plasmid containing full-length rat SK3 cDNA (SK3-pTracer-CMV2) and the empty vector (pTracer-CMV2; generous gifts from Dr. S. Lidofsky) were transfected into MCF-7 and 184A1 cells using LipofectAMINE 2000 (Invitrogen Life Technologies). Transfections were done according to the protocol of the manufacturer.

Immunocytochemistry

MDA-MB-435s were incubated with anti-SK3 antibody (1:100) followed by Alexa Fluor 488 nm goat anti-rabbit (1:1,000; Molecular Probes). Stained cells were viewed with an Olympus Fluoview 500 Instrument confocal microscope.

Solutions and Drugs

The physiologic saline solution in mmol/L: NaCl 140, $MgCl_2$ 1, KCl 4, $CaCl_2$ 2, D-glucose 11.1, and HEPES 10, adjusted to pH 7.4 with NaOH. The pipette solution for the whole-cell recording, pCa = 7 was (in mmol/L): K-glutamate 125, KCl 20, $CaCl_2$ 0.37, $MgCl_2$ 1, Mg-ATP 1, EGTA 1, HEPES 10, adjusted to pH 7.2 with KOH.

Tetraethylammonium (TEA), 4-aminopyridine (4-AP), and apamin were added to the physiologic saline solution or culture medium at the concentrations indicated in the figure legends. All drugs and chemicals were purchased from Sigma-Aldrich except Lei-Dab7, which was a generous gift from Dr. J.M. Sabatier.

Statistics

Unless otherwise indicated, data were expressed as mean ± SE (n = number of cells). Statistical analysis, done with StatView 4.57 software (Abacus Concepts, Berkeley, CA), was made using Student t test or one-way factor ANOVA followed by *post hoc* Bonferroni-Dunn test. Differences were considered significant when $P < 0.05$.

Results

Small-Conductance KCa Channels Are Involved in MDA-MB-435s Cell Migration by Regulating Membrane Potential

Cell migration, a key mechanism in epithelial tumorigenesis, has been found to be regulated by intracellular Ca^{2+}, which depends on the activity of K^+ channels. As a consequence of small-conductance KCa channel activity, we speculated that small-conductance KCa channels might be involved in the migratory ability of cancer cells, and we tested the effect of various blockers of small-conductance KCa channels on MDA-MB-435s cell migration. Apamin blocks SK2 and SK3 channels at low concentration (1 nmol/L), and does not affect SK1 and SK4 channels (19, 20). As shown in Fig. 1A, apamin treatment decreased the number of migrating cells without affecting cell proliferation/viability. A similar inhibitory effect was found using two general blockers of K^+ channels, 4-AP that blocks SK3 channel (21) and TEA that blocks both SK2 and SK3 channels (Fig. 1B; refs. 8, 22). In contrast, a specific SK2 channel blocker, Lei-Dab7 (23), had no effect on MDA-MB-435s cell migration (Fig. 1B). Because no specific SK3 channel blocker is available, we were unable to prove the involvement of SK3 by this approach.

To verify the modulation of intracellular Ca^{2+} concentration by small-conductance KCa channels inhibition, we measured it in MDA-MB-435s following 24 hours apamin

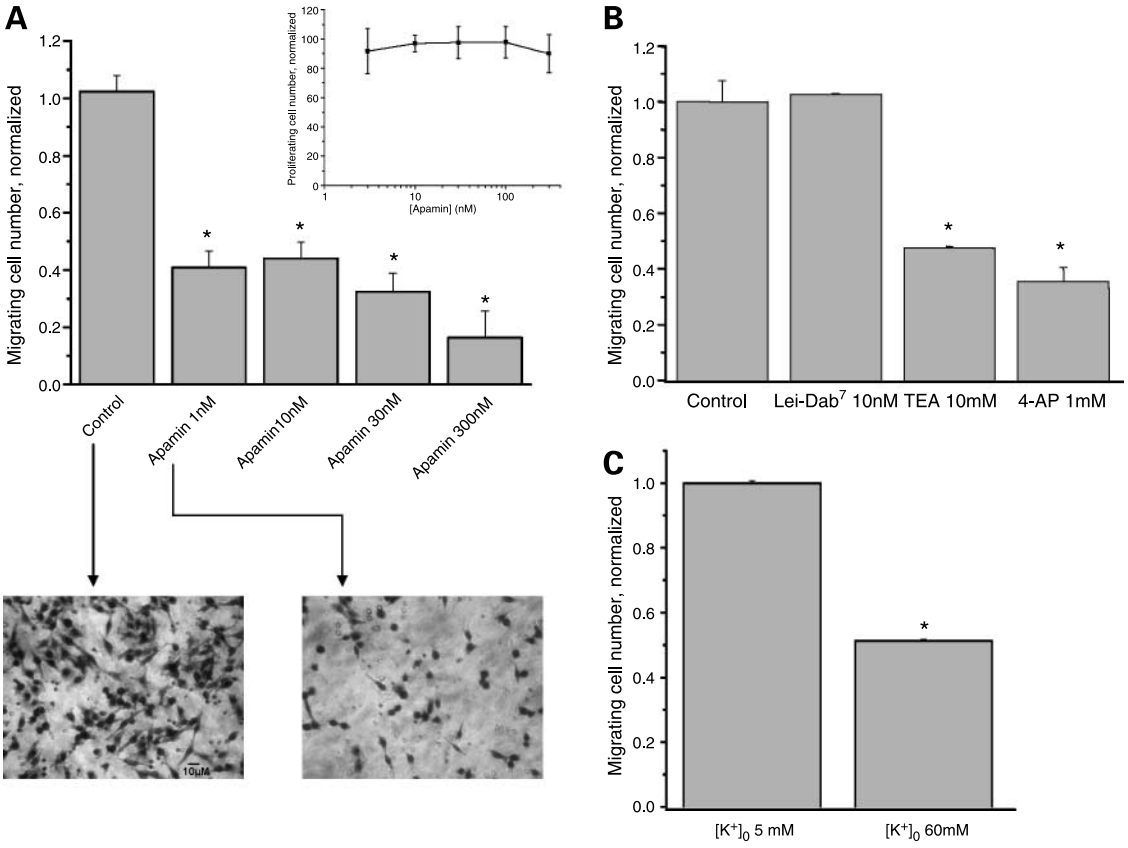

Figure 1. Involvement of SK channels in MDA-MB-435s cell migration. Effect of apamin (**A**), Lei-Dab7, TEA, 4-AP (**B**), and increasing concentration of external K^+ (**C**) on cell migration. Cells were seeded at 40,000 in a cell culture insert in DMEM with 5% fetal bovine serum ± drugs or [K^+] 60 mmol/L. The lower compartment of the insert contained DMEM with 10% fetal bovine serum as a chemoattractant ± drugs or [K^+] 60 mmol/L. After 24 h, cells of the lower compartment were stained with hematoxylin (**A**, *bottom*) and counted. The normalized cell number corresponded to the ratio of total number of migrating cells in presence of drug or [K^+] 60 mmol/L/total number of migrating cells in control experiments. The drug concentrations selected have no effect on cell proliferation and viability (example with apamin in **A**, *inset*). Bar, 10 μm for the two panels. From two separate experiments done in triplicate. *Columns,* mean; *bars,* SE. *, $P < 0.05$, significantly different from control.

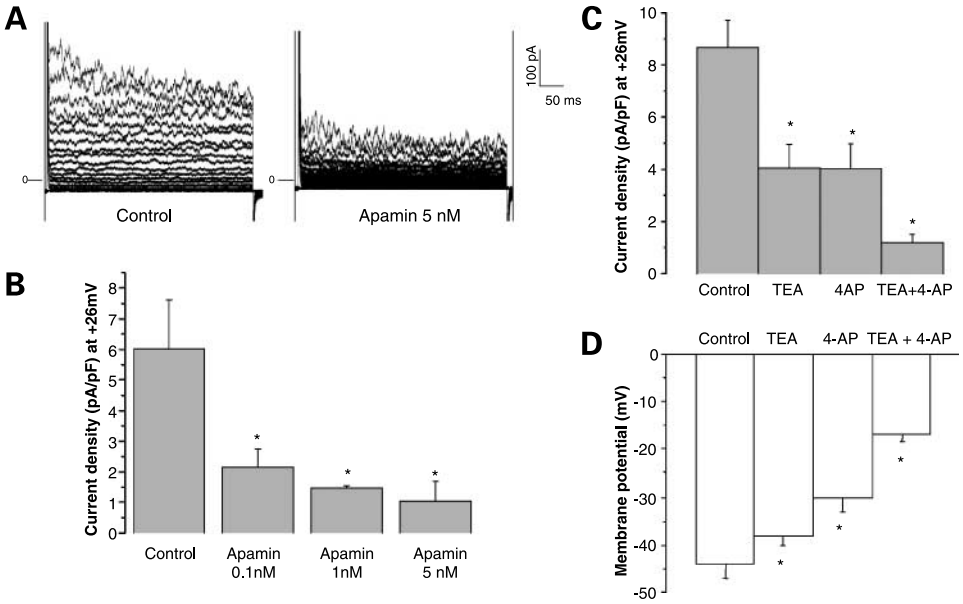

Figure 2. Regulation of resting membrane potential by SK channels in MDA-MB-435s cells. **A,** example of whole-cell macroscopic K^+ currents recorded in one cell without (control) or with apamin in the external medium. Currents were generated by stepwise 8-mV depolarizing pulses (400-ms duration; 5-sec intervals) from a constant holding potential of −70 mV up to +58 mV. Signals were filtered at 1 kHz and digitized at 10 kHz. **B** and **C,** current density was obtained by dividing the averaged steady-state current elicited at +26 mV (recorded during the latest 50 ms of the pulse) by the respective cell capacitance. Membrane capacitance was calculated by integrating the capacitive current measured during a 10-mV voltage step. *Columns,* mean of the inhibitory effects of apamin (n = 4), TEA (n = 7), 4-AP (n = 8), and TEA plus 4-AP (n = 3); *bars,* SE. **D,** variations of membrane potential recorded in control conditions (physiologic saline solution without drugs, n = 11) and in presence of TEA (n = 7), 4-AP (n = 8), and TEA plus 4-AP (n = 3). Membrane potential was measured in current-clamp mode (I = 0) just after the disruption of the patch membrane. *Columns,* mean; *bars,* SE *, $P < 0.05$, significantly different from control.

treatment. As expected, 10 nmol/L apamin (a concentration sufficient to block SK2 and SK3 channels) decreased basal intracellular Ca^{2+} concentration by 46% (from 379 ± 30 nmol/L, n = 28, to 204 ± 18 nmol/L, n = 34, $P < 0.05$), confirming that apamin-sensitive channels control intracellular Ca^{2+} concentration. Moreover, when MDA-MB-435s cells were cultured in the presence of high extracellular K^+ (60 versus 5 mmol/L in classic DMEM), the number of migrating cells decreased to a level close to that obtained with apamin, TEA, and 4-AP (Fig. 1C). Increasing extracellular K^+ changes K^+ equilibrium potential (E_K) from −86 to −22 mV (calculated using Nernst equation) and, as a consequence, leads to membrane depolarization.

To confirm that small-conductance KCa channels regulate membrane potential of MDA-MB-435s cells, we did patch-clamp experiments. Figure 2A shows typical examples of whole-cell outward currents recorded in MDA-MB-435s cells. These outward currents showed no apparent time dependence, which is one characteristic of all small-conductance KCa currents. To study the possible involvement of small-conductance KCa channels in these epithelial cells, we tested previously described blockers of small-conductance KCa channels (apamin, TEA, and 4-AP). As illustrated in Fig. 2A and B, apamin largely decreased MDA-MB-435s outward currents. The blocking effect of apamin, which is dose dependent, started at very low concentration, and estimated IC_{50} was lower than 0.1 nmol/L (Fig. 2B), suggesting that this apamin-sensitive current could be composed of SK3 and/or SK2 channels. Similarly, both TEA or 4-AP decreased the outward currents by 50% (Fig. 2C) and depolarized membrane of MDA-MB-435s cells (Fig. 2D). Interestingly, cotreatment with TEA and 4-AP led to an additive effect (Fig. 2C and D), suggesting that those two blockers do not act on the same small-conductance KCa channel subtypes. Finally, SK2 and SK3 channels both regulate membrane potential of MDA-MB-435s cells.

SK3 Protein Channel Is Expressed in MDA-MB-435s Cells and in Breast Tumor Tissues

To further investigate the role of SK2/SK3 channels in MDA-MB-435s cell migration, reverse transcription-PCR and Western blot analyses were done. Central nervous system tissues, known to highly express small-conductance KCa channels, particularly SK3 channel, were used as a positive control (24). As shown in Fig. 3A and B, MDA-MB-435s expressed both SK2 and SK3 channels. These experiments confirm that apamin-sensitive outward currents observed in these epithelial cancer cells were due to activation of SK2 and SK3 channels. *SK1* gene expression was found in human central nervous system but not in MDA-MB-435s (Fig. 3A). In contrast, *SK4* gene expression was found in MDA-MB-435s but only slightly in human central nervous system (Fig. 3A). This is in agreement with other works showing that if SK1 is principally expressed in

central neurons, *SK4* expression is restricted outside of the brain (10).

We next examined whether SK2/SK3 proteins are also expressed in immortalized (MCF-10A, 184A1) or in cancerous (MDA-MB-231, T47D, SKBR3) mammary epithelial cell lines. As shown in Fig. 3B, SK2 was expressed in all cell lines. In contrast, SK3 protein was solely expressed in MDA-MB-435s cells and exhibited a membrane location as shown by immunocytochemical experiments (Fig. 3C).

All the immortalized (MCF-10A, 184A1) or cancerous (MDA-MB-231, T47D, SKBR3) mammary epithelial cells, which exhibit low migrating capacity compared with MDA-MB-435s, are insensitive to apamin treatment (data not shown), thus confirming that SK3 involvement, but not the sole expression of SK2, is necessary to MDA-MB-435s migration process.

Finally, we analyzed the protein expression of SK2 and SK3 channels in tumor and nontumor breast tissues. As SK3 and SK2 antibodies were unsuitable for immunohistochemical experiments, we were unable to test for the presence of small-conductance KCa channels in frozen or paraffin-embedded biopsies. Using Western blot analyses, we found that, as observed in mammary epithelial cancer cells, SK3 was only observed in tumor breast biopsies whereas SK2 protein was found in both tumor and nontumor breast tissues (Fig. 3D).

Whereas *SK3* Gene Transcript Destruction Decreased Migration of MDA-MB-435s, Enforced *SK3* Gene Expression Increased Migration of MCF-7 and 184A1 Cells

To fully show the contribution of SK3 protein to MDA-MB-435s migration, SK3 mRNA was knocked down by transiently transfecting cells with two different sets of siRNA locating in exon 1 (Δexon1) or exon 3 (Δexon3) of *SK3* human gene, or with scrambled siRNAs as a negative control. Western blot analysis and *in vitro* cell migration test were done 24, 48, and 72 hours after siRNAs transfection. Figure 4A (*top*) shows a marked suppression of SK3 expression in cells after transfection with both SK3-siRNAs, when compared with cells transfected with scrambled siRNA, with the greatest effect observed at 72 hours. As expected, the knockdown of SK3 markedly reduced the number of MDA-MB-435s migrating cells (Fig. 4A, *bottom*). Note that, as observed with the Western blot, Δexon3 siRNA was more efficient than Δexon1 siRNA. The reason why efficiency is different remains to be elucidated. Previous reports described several *SK3* mRNA variants with distinct sequences for exon 1 that encode SK3 proteins with distinct NH_2 termini (25, 26). It is not excluded that high Δexon3 siRNA efficiency is due to the full targeting and silencing of SK3 isoforms, in contrast to Δexon1 siRNA. Nevertheless, both siRNAs are specific to SK3 protein

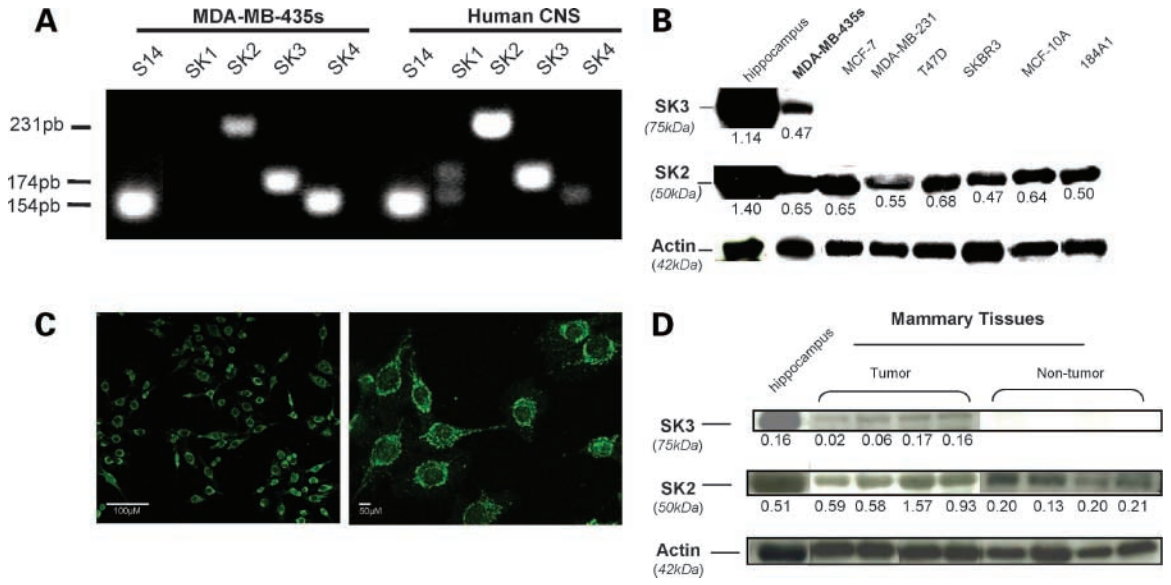

Figure 3. SK3 protein is expressed in MDA-MB-435s and in tumor breast tissue. **A,** detection of SK channels (SK1, SK2, SK3, and SK4) and of the housekeeping ribosomal S14 mRNA in MDA-MB-435s. Reverse transcription-PCR was done in MDA-MB-435s cells and in human central nervous system cDNA as a positive control. Primers used for the reverse transcription-PCR experiments are listed in Materials and Methods. Representative examples of three separate experiments. **B,** representative Western blot pattern of SK2 and SK3 protein expression in cancerous and noncancerous mammary epithelial cell lines. Lysates of human mammary cancer cell lines (MDA-MB-435s, MDA-MB-231, MCF-7, T47D, and SKBR3), of noncancerous mammary epithelial cell lines (184A1, MCF-10A), and of rat hippocampus tissue (used as positive control) were prepared in lysis buffer (SDS 5%, protease inhibitors 1%, phenylmethylsulfonyl fluoride 200 mmol/L). Cell extracts were subjected to electrophoresis on SDS-polyacrylamide gel under reducing conditions and the signal was detected by enhanced chemiluminescence. Results were provided in triplicate. The numbers represent the SK2 and SK3 band absorbance normalized to those obtained with actin. **C,** confocal pictures showing the cellular location of SK3 channel in MDA-MB-435s cells. Cells were fixed and permeabilized with 100% methanol before antibody staining. **D,** Western blot pattern showing the expression of SK2 and SK3 proteins in tumor ($n = 4$) and nontumor ($n = 4$) breast biopsies. The numbers represent the SK2 and SK3 band absorbance normalized to those obtained with actin.

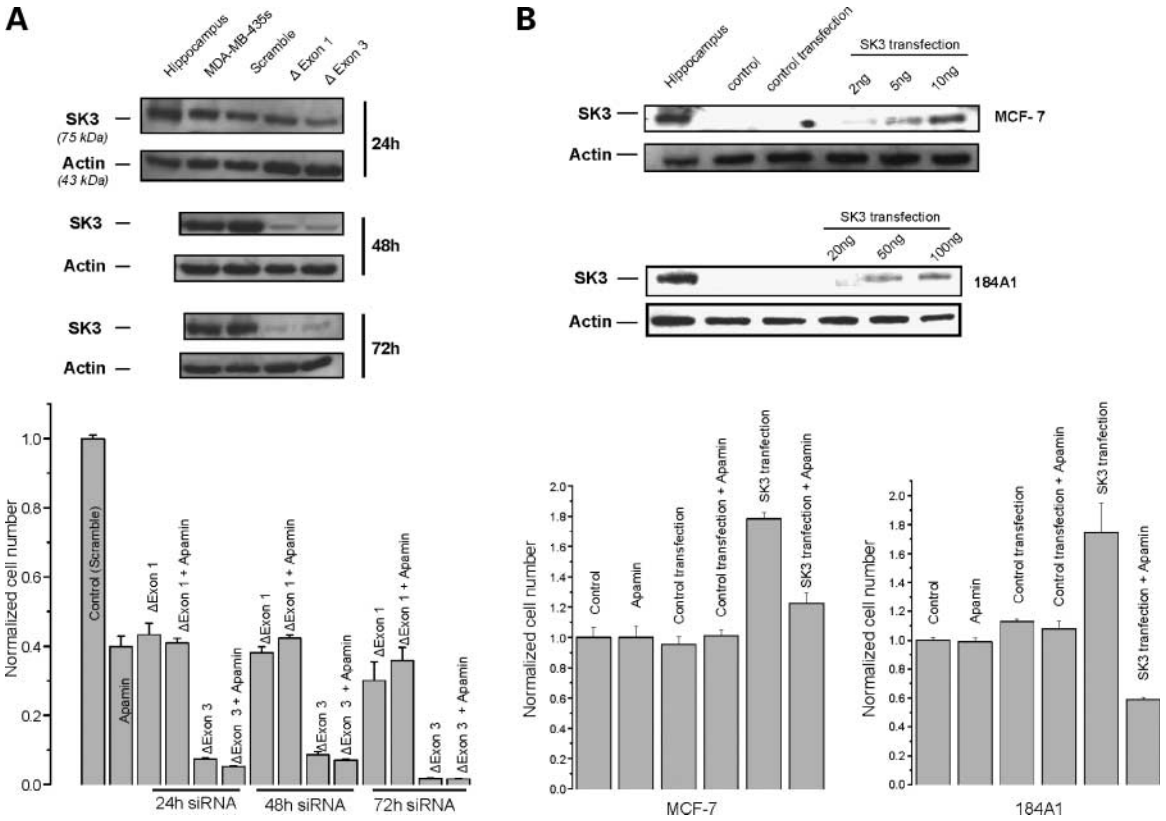

Figure 4. *SK3* gene transcript destruction decreases migration of MDA-MB-435s cells, and *SK3* gene expression increases migration of 184A1 cells. **A**, *top*, Western blot patterns showing the silencing effect on the expression of SK3 protein of two siRNAs designed against SK3 mRNA. Cells were transfected with siRNA-LipofectAMINE complexes for 24, 48, and 72 h. A scrambled siRNA was used as negative control. siRNA oligonucleotide sequences are listed in Materials and Methods. *Bottom*, histograms showing the inhibitory effect on MDA-MB-4345s cell migration after 24, 48, and 72 h of siRNA transfection with or without 10 nmol/L apamin. Results from two separate experiments done in triplicate. *Columns,* mean; *bars,* SE. Normalization of cell number done as described in Fig. 1. Note that the cells have lost their sensitivity for apamin after siRNA transfection, indicating a specific effect on SK3 protein. **B**, *top,* Western blot patterns showing the expression of the SK3 protein channel after transient transfection of SK3-pTracer-CMV2 plasmid (SK3 transfection) or empty vector (control transfection) in cancerous (MCF-7) and noncancerous (184A1) mammary epithelial cell lines. *Bottom,* histograms showing the number of migrating cells after transient transfection, with or without 10 nmol/L apamin. Results from two separate experiments done in triplicate. *Columns,* mean; *bars,* SE Note that SK3-transfected cells have gained a sensitivity to apamin, indicating a specific expression of SK3 channel.

channel, as the residual migrating capacity of cells is unaffected by apamin (Fig. 4A, *bottom*). Our data further show that endogenous SK3 channel is necessary for MDA-MB-435s migration.

To further validate this unusual physiologic activity of SK3 channel, we wondered whether enforced SK3 expression might promote migratory capacity to cells lacking the SK3 channel. We addressed this question by transiently transfecting SK3 in MCF-7 and 184A1 cell lines. As shown in Fig. 4B, overexpression of the SK3 channel increased the number of migrating cells. Furthermore, upon treatment with the SK3 inhibitor apamin, the number of migrating cells was markedly decreased, strengthening the observed association between SK3 and cell migration capacity.

Discussion

This study shows, for the first time, that SK3 protein is expressed in a highly metastasizing mammary cancerous cell line, MDA-MB-435s, and in tumor breast biopsies but not in nontumor breast biopsies. The use of a panel of small-conductance KCa blockers together with siRNA or overexpression approaches led us to show that SK3 channel are involved in cell migration but not in cell proliferation. Thus, SK3 channel seems to be a new mediator of breast cancer cell migration and possibly a novel therapeutic target against epithelial tumor growth or metastasis, two main clinical expressions of tumor cell migration.

SK3 Channels Promote Epithelial Cell Migration by Increasing Intracellular Ca^{2+} Concentration

Cancerous mammary epithelial cell line MDA-MB-435s possess apamin-sensitive small-conductance KCa channels that regulate resting membrane potential as already described in central nervous system and smooth muscle (9, 11–13). We found that SK3 channel is necessary and promotes cancerous mammary epithelial cell migration by hyperpolarizing their plasma membrane. Although SK3 channel-induced hyperpolarization decreases intracellular

Ca^{2+} concentration in excitable tissues, SK3 channels maintained a high level of intracellular Ca^{2+} concentration in MDA-MB-435s. This could be explained by the presence of voltage-independent Ca^{2+} channels through which Ca^{2+} entry increases following membrane hyperpolarization (27, 28). The increase of epithelial cell migration mediated by SK3 channel would therefore be the result of an increased K^+ efflux and subsequent shift of the membrane potential to more negative values, leading to Ca^{2+} entry through voltage-independent Ca^{2+} channels (Fig. 5).

SK3 Channel Is a Low-Energy Cost and a High Efficiency Mediator of Cell Migration

Many of the key proteins involved in cell migration, including enzymes (3, 4) and actomyosin cytoskeleton (29), can be regulated by intracellular Ca^{2+}. However, in most cases, it is unknown how the increase of intracellular Ca^{2+} is generated. Some ionic channels and transporters are involved in cancer cell migration and can directly or indirectly allow Ca^{2+} entry (14, 30). Nevertheless, the role of small-conductance KCa has never been observed. We found that SK3 protein, which is differentially expressed in breast tumor and nontumor tissues, promotes mammary epithelial cell migration. Then, in SK3-expressing cells, the intracellular Ca^{2+} concentration may be elevated using the electrochemical driving force generated by K^+ efflux through SK3 channels at no energy cost to increase Ca^{2+} entry through existing plasma membrane voltage-independent Ca^{2+} channels (27, 28). Therefore, a positive feedback loop exists in which Ca^{2+} entry through Ca^{2+} channels increases the activity of SK3 channel, leading to a more negative membrane potential, which result in a stronger electrochemical driving force for Ca^{2+} and an enhancement of Ca^{2+} entry through voltage-independent Ca^{2+} channels.

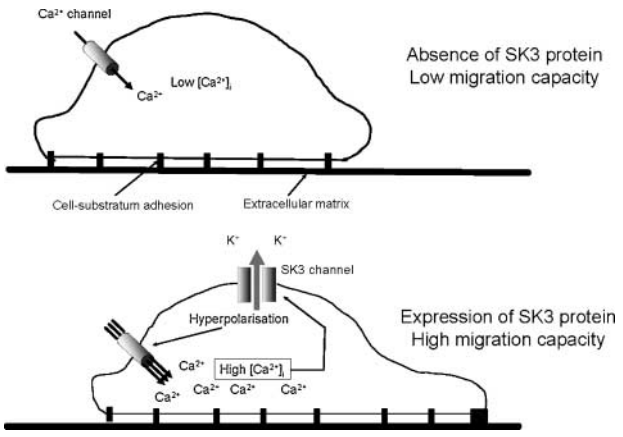

Figure 5. Proposed model to explain how SK3 channel promotes epithelial cell migration. *Top,* when SK3 channel is not present, plasma membrane is depolarized (−30 to −20 mV) and Ca^{2+} influx through voltage-independent Ca^{2+} channels is low. As a consequence, intracellular Ca^{2+} concentration is low and epithelial cell have a low migration capacity. *Bottom,* expression of SK3 leading to SK3 channel activity would result in an increase of K^+ efflux. This leads to a shift of membrane potential of epithelial cells to more negative values (hyperpolarization to −50 mV), which is equivalent to a stronger electrochemical driving force supporting Ca^{2+} entry through voltage-independent Ca^{2+} channels. This Ca^{2+} entry would increase intracellular Ca^{2+} concentration that promotes epithelial cell migration. Like a positive feedback loop, Ca^{2+} entry would also increase the activity of SK3 channel, leading to a more negative membrane potential.

Recently, Brainard et al. (31) showed that, in smooth muscle, KCa channels localize to caveolae close to the cytoskeleton to form an actin-KCa channel-caveolin microdomain complex. According to this finding and because the generation and control of cell polarity is a fundamental mechanism for directed cell migration, it would be interesting to test whether SK3 channel complexes involved in cell migration display a specific plasma membrane location in cancer epithelial cells. All these features make SK3 channel as an economical and efficient mediator of cancer cell migration.

Should SK3 Channel Exhibit a Specific Association to be Effective in Cell Migration?

Numerous reports have suggested the possibility that SK3 protein forms heteromeric channel with the other small-conductance KCa channel subunits SK1 and SK2 (7, 8). Because *SK1* gene is not expressed in MDA-MB-435s cells, assembly of either SK3 proteins or SK3 and SK2 proteins, resulting in SK3 homomeric or SK2/SK3 heteromeric channels, respectively, may make the functional small-conductance KCa channel more or less involved in cell migration. The possible involvement of heterotetrameric association of SK2/SK3 proteins could explain the discrepancy between 184A1 and MCF-7 cells in their response to SK3 overexpression. In contrast to SK3-transfected MCF-7 cells in which apamin reduced the number of migrating cells to reach the number of nontransfected migrating cells, in noncancerous epithelial cells 184A1, apamin reduced cell migration to a lower level than those of nontransfected cells. This discrepancy may be explained by a difference in SK2 protein level expression (see Fig. 3B), which agrees with the involvement of SK2/SK3 heteromeric association. Further experiments are necessary to determine the precise structure of the functional SK3 channel effective in cell migration.

In conclusion, the novel SK3 function presented here, taken together with the intrinsic SK3 channel expression in human breast cancer, suggests that the SK3 channel may become a novel therapeutic target and/or new molecular marker of breast epithelial tumor. As a next step, it will be important to determine whether expression of the SK3 channel affects epithelial tumor metastasis and, if so, to develop and test specific SK3 blockers.

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