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Effects of Pharmacological Modulators of Ca^{2+}-activated K^+ Channels on Proliferation of Human Dermal Fibroblast

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Employing electrophysiological and cell proliferation assay techniques, we studied the effects of Ca^{2+}-activated K^+ channel modulators on the proliferation of human dermal fibroblasts, which is important in wound healing. Macroscopic voltage-dependent outward K^+ currents were found at about −40 mV stepped from a holding potential of −70 mV. The amplitude of K^+ current was increased by NS1619, a specific large-conductance Ca^{2+}-activated K^+ (BK) channel activator, but decreased by iberiotoxin (IBTX), a specific BK channel inhibitor. To investigate the presence of an intermediate-conductance Ca^{2+}-activated K^+ (IK) channels, we pretreated the fibroblasts with low dose of TEA to block BK currents, and added 1-EBIO (an IK activator). 1-EBIO recovered the currents inhibited by TEA. When various Ca^{2+}-activated K^+ channel modulators were added into culture media for 1~3 days, NS1619 or 1-EBIO inhibited the cell proliferation. On the other hand, IBTX, clotrimazole or apamin, a small conductance Ca^{2+}-activated K^+ channel (SK) inhibitor, increased it. These results suggest that BK, IK, and SK channels might be involved in the proliferation of human dermal fibroblasts, which is inversely related to the channel activation.

Key Words: Ca^{2+}-activated K^+ channels, Cellular proliferation, Human dermal fibroblasts

INTRODUCTION

Fibroblasts constitute a predominant cell type in skin dermis, and they not only produce and organize the extracellular matrix, but also communicate with each other and other cell types, playing a crucial role in regulating skin physiology. The alterations of dermal matrix contribute to skin aging characterized by wrinkles and wound healing. Wound healing includes a series of overlapping phase of inflammation, cell proliferation, matrix deposition, and tissue remodeling (Luo & Chen, 2005). Loss of functional healing process could lead to severe disabilities such as decubitus ulcers, venous ulcers, and diabetic ulcers.

Ion channels have recently been shown to contribute to the regulation of cell proliferation. Inhibition of K^+ channel function leads to a decrease of proliferation in both physiological responses and pathological condition (Wonderlin & Strobl, 1996). Nevertheless, only a few types of K^+ channels have been identified to correlate with cell proliferation; for example, voltage-dependent K^+ channel, Herg channel, Eag1 and TASK-3 channel (Pardo, 2004).

Several families of Ca^{2+}-activated K^+ channels, that are prominent in mammalian cells, constitute a major link between second messenger systems and the electrical activity of the cells. On the basis of their electrophysiological characteristics, three major classes of Ca^{2+}-activated K^+ channels have been described: large-conductance channels (BK), intermediate-conductance channels (IK), and small-conductance channels (SK). BK channels in particular have extensively been studied, and found to play important roles in membrane repolarization during action potential, neuronal firing rates, and neurotransmitter release control in excitable cells (Weiger et al, 2002). BK channels are also important in microbicidal function of neutrophils (Ahluwalia et al, 2004), in differention of keratinocytes (Kogel & Alzheimer, 2001) and in proliferation of endothelial cells (Wiecha et al, 1998). BK channels have already been identified in human dermal fibroblasts (Estacion, 1991a; Goodwin et al, 1998; Lim et al, 2005). Unlike BK channels, IK channels are expressed exclusively in non-excitable cells, including endothelial cells, epithelial cells, immature smooth muscle cells, lymphocytes, and erythrocytes (Jensen at al, 2001), and they are now considered as important regulators of cell proliferation, since up-regulation of these channels have been shown to be an essential step in the mitogenesis of various cells (Jager et al, 2004; Ouadid-Ahidouch et al, 2004; Grgic et al, 2005). However, the existence of IK channels has not yet been reported in human dermal fibroblasts. 1-ethyl-2-benzimidazolinone (1- EBIO) and riluzole, a specific IK channel opener, increase prostate cancer cell proliferation (Parihar et al, 2003). SK channels may exist in human dermal fibroblasts, since apamin

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ABBREVIATIONS: BK channel, large-conductance Ca^{2+}-activated K^+ channel; IK channel, intermediate-conductance Ca^{2+}-activated K^+ channel; SK channel, small conductance Ca^{2+}-activated K^+ channel; IBTX, iberiotoxin; 1-EBIO, 1-ethyl-2-benzimidazolinone.

inhibits a calcium ionophore, ionomycin-induced K^+ current modulation in bradykinin-stimulated human fibroblasts (Estacion, 1991b). However, little is known concerning the role of various types of Ca^{2+}-activated K^+ channels in dermal fibroblasts proliferation.

In the present study, the existence of Ca^{2+}-activated K^+ channels and the effects of their pharmacological modulators on cellular proliferation were examined in human dermal fibroblasts.

METHODS

Cell preparation and culture

Normal penile skin tissues, the use of which was approved by the Institutional Review Board, were obtained from 10~13 year old boys during circumcision. The skin explants were washed with phosphate-buffered saline (PBS, GIBCO, Grand Island, NY) solution, and the epidermal layer was excised. The minced dermal tissues were transferred to a solution composed of Dulbecco's modified Eagle's medium (DMEM, GIBCO) and PBS (1 : 1). After centrifugation, the tissues were agitated and transferred to culture medium containing DMEM, fetal bovine serum (FBS, 20%, GIBCO, Grand Island, NY), and penicillin (1%, Sigma, St. Louis, MO). Dissociated cells were incubated in a 5% CO_2 incubator at 37°C until confluence, and the resulting fibroblasts were detached with trypsin (0.05%, Sigma, St. Louis, MO) and EDTA (0.02%, Sigma, St. Louis, MO) in DMEM, and then serially passed into 100 mm culture dishes. Cells at the 4^{th} passage of culture were frozen in cell culture freezing medium, and stored in liquid nitrogen. Before experiments, the cells were thawed and cultured in DMEM with FBS (10%). When the 5^{th} passage cells achieved confluence, the cells were washed with DMEM, and treated with trypsin/EDTA in DMEM. After 3~5 min of incubation in a 5% CO_2 incubator (37°C), the cells were suspended in fresh DMEM and centrifuged again. The supernatant was then discarded, and the diluted cell suspension (1 : 10 with DMEM) was seeded onto poly-L-lysine coated cover slips for experiments.

Electrophysiological recordings

Electrophysiological measurements were taken using the tight-seal patch clamp method. Whole cell mode recording was performed with an Axopatch 200B patch clamp amplifier (Axon Instruments, Union City, CA). After achieving the whole-cell configuration, cells were allowed to stabilize and dialyzed for 5 min before the start of recording. Whole-cell currents were filtered at 2 kHz and digitized at 10 kHz. Cells were clamped at −70 mV, and depolarizing pulses were applied in 10 mV steps to evoke outward K^+ currents. pCLAMP 9.0 (Axon Instruments) software was used for data acquisition as well as the analysis of the currents. Recording pipettes were pulled from borosilicate glass capillaries (TW150F-4, World Precision Instruments, Sarasota, FL) using a microelectrode puller (PP-83, Narishige, Japan), and then fire polished on a microforge (MF-83, Narishige, Japan). After fire polished, the pipettes exhibited a resistance of 2~3 MΩ in whole-cell recordings when filled with pipette solution. All experiments were carried out at room temperature (21~23°C).

Whole cell recordings were performed in physiological solution in the bath (mM); 145 NaCl, 5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 5 glucose, 5 HEPES (adjusted to pH 7.35 with NaOH). Pipette solution contained (mM); 145 KCl, 1.652 $CaCl_2$ (pCa 6.0), 1.013 $MgCl_2$, 10 HEPES, 2 EGTA, 2 K-ATP (pH 7.3).

Other chemicals including 4-(2-hydroxyethyl)-1-piperazineethane sulphonic acid (HEPES), iberiotoxin (IBTX), and 1-ethyl-2-benzimidazolinone (1-EBIO) were purchased from Sigma (St. Louis, MO). Apamin and clotrimazole were dissolved in dimethyl sulfoxide (DMSO), and the concentration of DMSO was less than 0.1%.

Cell proliferation assay

The confluent fibroblasts of the 4th passage cultures were trypsinized (0.25% trypsin/0.02% EDTA) and seeded at a density of 2,500~3,000 cells/well in 96-well plates. After 24 hr, old medium was replaced with fresh medium. The incubation medium was modified by adding various chemicals (NS1619, iberiotoxin, 1-EBIO, clotrimazole, and apamin) according to experimental settings. The modified medium was replaced every 2 days. In all cases, they were freshly prepared in DMEM supplemented with 10% FBS immediately before use. Cell cultures were incubated for 6~72 hr in a humidified atmosphere of 95% air and 5% CO_2.

Ten μl of Cell Counting Kit-8 (CCK-8) solution (Dojindo, Japan) was added to each well and incubated for 2 hr in 95% air and 5% CO_2 incubator. The optical density of each well was measured 450 nm, using a Spectra Max 340 ELISA reader (Molecular devices, USA) with a reference wavelength at 600 nm. Tetrazolium salt (WST-8) of CCK-8 solution produces a formazan dye, and the amount of yellow colored formazan dye generated by dehydrogenase in the fibroblasts is directly proportional to the number of viable cells in a culture medium. The optical density of control group was taken as 100% viable cells.

Statistical analysis

Data are expressed as means±standard error of mean (SEM). Comparisons of measurements between groups were conducted using the Student's *t*-test, depending on the experimental design. A significant level of difference was set at either $p<0.05$ or $p<0.01$.

RESULTS

Whole-cell mode patch clamp recordings were performed on human dermal fibroblasts. Macroscopic K^+ currents were generated by incremental 10 mV depolarizing steps from −60 mV. The holding potential was −70 mV. Outward currents were activated at about −40 mV and well maintained throughout the test pulse. The K^+ currents were increased with depolarizing stimulation and oscillated at strong depolarization. The current-voltage (I~V) relationship exhibited strong outward rectification. The K^+ currents were increased by NS1619 (a specific BK channel activator) up to 75.5±5.23% above the control (at 50 mV, n=4, $p<0.01$, Fig. 1). On the other hand, addition of IBTX (100 nM, a specific BK channel inhibitor) attenuated the currents by 46.3±4.5% (at 50 mV, n=4, $p<0.01$, figure not shown). These results indicate that the K^+ currents detected with this configuration was mainly mediated by the

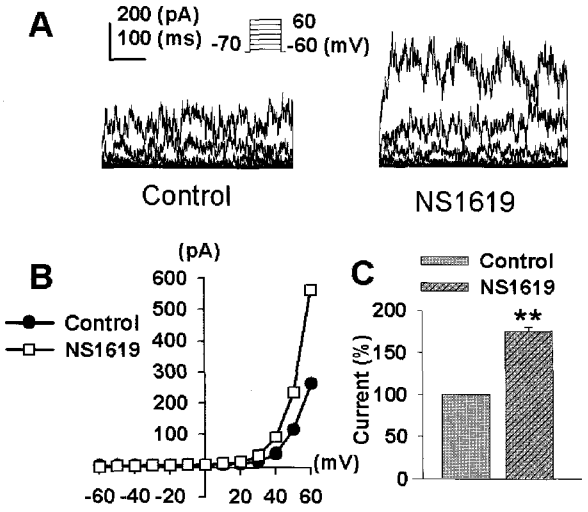

Fig. 1. Identification of BK current in human skin fibroblasts by whole-cell mode patch clamp technique. Holding potential was −70 mV. (A) Representative effect of NS1619 (a specific BK channel activator) on the outward K^+ currents. (B) I~V relationship for steady state outward K^+ currents (C) The bar graph shows the current amplitude at 50 mV. $^{**}p<0.01$ compared with control.

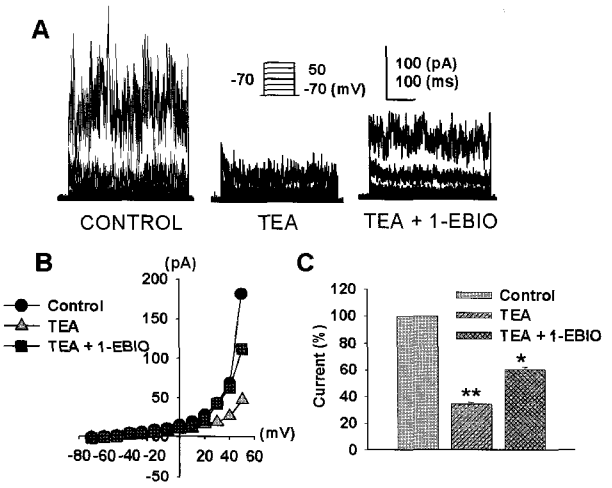

Fig. 2. Isolation of IK current in human skin fibroblasts. (A) Representative currents in the presence of TEA (1 mM) or TEA plus 1-EBIO (B) I~V curves for steady state currents. (C) The bar graph shows the current amplitude at 50 mV. $^{*}p<0.05$, $^{**}p<0.01$ compared with control.

opening of BK channels.

We next investigated the presence of IK channel in human dermal fibroblasts. At first, we tested low concentration of tetraethylammonium chloride (TEA), that is well known to selectively inhibit BK channel at this concentration, and IBTX. There was no significant difference in the inhibition of the currents between TEA and IBTX; TEA (1 mM) significantly inhibited the outward K^+ currents (34.5±2.40% as compared with 100% of control at 50 mV, n=10, $p<0.01$), and IBTX (100 nM) also inhibited the K^+

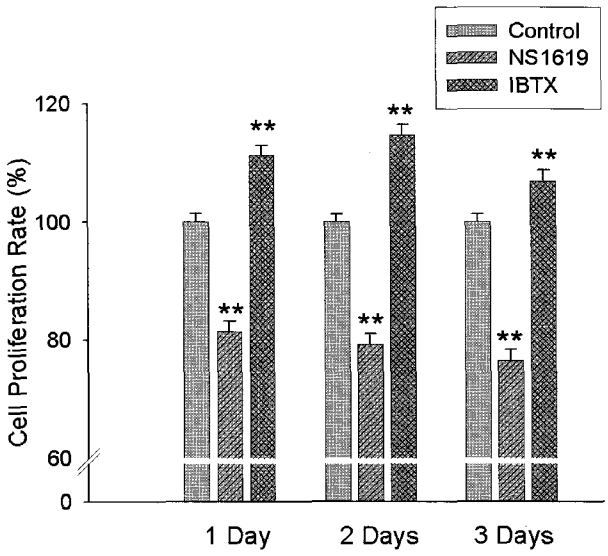

Fig. 3. Effects of BK channel modulators on the proliferation of human dermal fibroblast. NS1619 (50 μM) significantly inhibited cell proliferation, but IBTX (100 nM) increased cell proliferation ($^{**}p<0.01$ from control at the indicated number of days). The control response was set as 100%, which is represented by optical density of unstimulated cells. Values are expressed as % of cell proliferation. Data presented are the average of observations in 40 wells (2,500~3,000 cells/well).

currents (38.2±3.21% as compared with control at 50 mV, n=10, $p<0.05$). After preincubation of cells with 1 mM TEA, 300 μM 1-EBIO was added to activate IK current, and the current was recovered to 60.4±3.17% of the control (at 50 mV, n=9, $p<0.05$, Fig. 2). The results show that IK as well as BK constitutes the outward K^+ currents in human dermal fibroblasts.

The role of BK channels in the proliferation of human dermal fibroblast was investigated. Data presented are the average of observations in 40 wells (2,500~3,000 cells/well). When NS1619 (50 μM), a specific BK channel activator, was added to the culture medium, the proliferation of the cells was significantly blocked (81.4±1.75% on the 1st day, 79.2±1.89% on the 2nd days, and 76.5± 1.89% on the 3rd days compared to control, n=40, $p<0.01$, Fig. 3). On the other hand, IBTX (100 nM) increased the cellular proliferation significantly (111.2±1.71% on the 1st day, 114.7±1.74% on the 2nd days, and 106.8±1.92% on the 3rd days compared to control, n=40, $p<0.01$, Fig. 3). To further assess the role of other Ca^{2+}-activated K^+ channels, the effects of other IK and SK channel modulators were also investigated. 1-EBIO (500 μM), a specific IK channel opener, significantly inhibited the cellular proliferation (92.5±1.52% on the 1st day, 84.4±1.47% on the 2nd days, and 73.3±1.40% on the 3rd days, n=40, $p<0.01$, Fig. 4). Clotrimazole (2 μM), a specific IK blocker, increased the cell proliferation significantly (compared to 100% of control, 115.1±1.69% on the 1st day, 116.3±1.75% on the 2nd days, and 108.8±2.17% on the 3rd days, n=40, $p<0.01$, Fig. 4). Apamin (a specific SK channel blocker, 0.5 μM) also increased the cell proliferation (111.6±1.67% on the 1st day, 113.1±1.73% on the 2nd days, and 107.7±1.83% on the 3rd days, n=40, $p<0.01$, Fig. 4). To dissolve clotri-

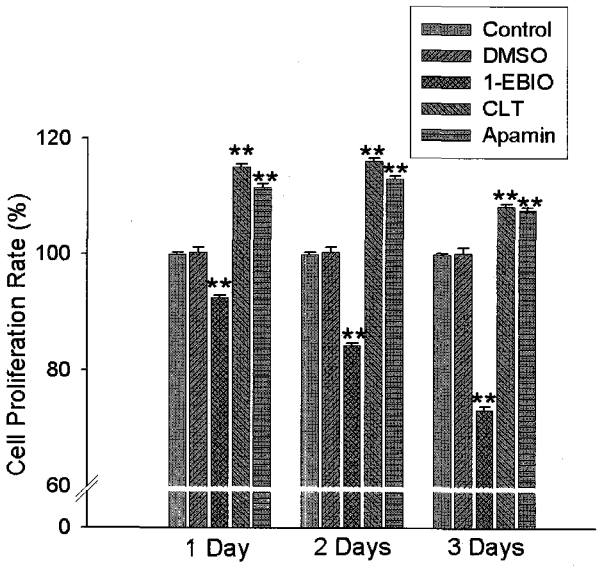

Fig. 4. Effects of IK channel and SK channel modulators on human dermal fibroblast proliferation. 1-EBIO (500 μM) significantly inhibited cell proliferation. Clotrimazole (CLT, 2 μM) and apamin (0.5 μM) increased cell proliferation (**$p<0.01$). The control response was set as 100%, which is represented by optical density of unstimulated cells. No cytotoxic effects was seen in proliferating cell by DMSO (0.5%). Data presented are the average of observations in 40 wells (2,500~3,000 cells/well).

mazole and apamin, DMSO was used, however, DMSO alone did not inhibit the proliferation of the cells at 0.5 and 1.0% concentrations (100.4±1.21 and 100.35±1.14 respectively, n=16).

DISCUSSION

In the present study, we found novel and unexpected findings that BK and IK channel openers decreased the proliferation of human dermal fibroblasts, while the blockers of various types of Ca^{2+}-activated K^+ channels showed opposite effects. It is highly likely that K^+ channels are important in proliferation process as key players in controlling membrane potential. Activation of K^+ channels is associated with the regulation of cell proliferation and progression of cell cycle, because transient hyperpolarization is required for the progression of the early G1 phase of the cell cycle (Wonderlin & Strobl, 1996). The hyperpolarization provides an electrochemical gradient for influx of Ca^{2+}, a messenger in the mitogenic signal cascades of cells. Thus, blockade of K^+ flux, which leads to depolarization is suspected to interfere with proliferation by inhibiting of such transient hyperpolarization. Ca^{2+}-influx is also well established as a crucial factor for cell proliferation in melanoma cells (Nilius et al, 1992). Furthermore, proliferation is also associated with volume increase along the G1 phase, but nonspecific cell swelling can inhibit proliferation (Lang et al, 2000). Certainly, changes in both membrane potential and cell volume are necessary for progression of the cell cycle, and both require the action of K^+ channels (Pardo, 2004). Activation of BK channels has been shown to be important in basic fibroblast growth factor (bFGF)-induced endothelial cell proliferation (Wiecha et al, 1998). Our present results also showed that BK channel is important in the proliferation of human dermal fibroblasts.

We could record IK currents using 1-EBIO activation after blocking of BK channels with IBTX or TEA in human native dermal fibroblasts. IK channels have also been showed to correlate with cell proliferation. Stimulation of T lymphocytes with mitogens results in increased IK channels density (Ghanshani et al, 2000), and the mitogenic action of basic fibroblast growth factor is linked to upregulation of IK channels in myogenic fibroblast cell lines (Pena & Rane, 1999). Thus, IK channels might prove to be a prime drug target to manipulate the mitogenic behavior of non-excitable cells. However, its physiological role is conceptually very difficult to separate from that of delayed rectifier K^+ currents in lymphocytes (Chandy et al, 2004) and inward rectifier K^+ currents in melanoma cells (Lepple-Wienhues et al, 1996). In the present study, 1- EBIO, a specific IK channel opener, was found to inhibit the cell proliferation. On the other hand, clotrimazole, an IK channel blocker, increased the proliferation. 1-EBIO and the other IK openers diminish mRNA levels and the activity of IK channels in HaCaT lymphocytes and C6 glioma cell lines, although they produce a prominent hyperpolarization. Anti-proliferative stimuli such as elevated Ca^{2+} and vitamin D also increase IK mRNA expression, and 1-EBIO inhibits the proliferation of human keratinocytes (Manaves et al, 2004). However, these authors failed to detect IK channel currents in keratinocytes by patch clamp techniques, suggesting that IK channel does not contribute much to K^+ currents in human keratinocytes.

We did not use SK channel activator in the present experiment. However, 1-EBIO also activates SK channels (Cao et al, 2001) as well as IK channels. Thus, we tested apamin (a specific SK channel blocker) and found that it increased the cell proliferation, suggesting that SK channel is also involved in the fibroblast proliferation similar to BK or IK channels. SK channel is recorded only in ionomycin augmented K^+ current (Estacion, 1991b), which means that SK is not abundant in human dermal fibroblast under physiological conditions. We do not yet know proportions of each channel's contribution to the proliferation of fibroblasts.

Our present data showed novel effects of Ca^{2+}-activated K^+ channels on the proliferation of human dermal fibroblasts which is unexpected and contrary to previous reports on other cell types (Wonderlin & Strobl, 1996; Wiecha et al, 1998). Rouzaire-Dubois et al. (2000) proposed two possible mechanisms that might account for this highly inverse relationship: changes of cell volume that may alter the concentration of cellular components involved in the expression or activity of cell cycle regulating proteins, and cytoskeleton rearrangements due to cell volume changes that may affect the protein kinases or phosphatases responsible for the control of cell cycle progression. Therefore, more experiments appear to need in order to demonstrate the relationship between the cell volume change and fibroblast proliferation rate. IK channel openers are presently considered as potentially beneficial agents in cystic fibrosis, chronic obstructive pulmonary disease, and others (Jensen et al, 2001; Kohler R et al, 2003). Our data suggest that K^+ channel openers might potentially be used as a pharmacological alternative to IK channel blockers, similar to clotrimazole in the treatment of epithelial and endothelial

hyperproliferative disorders, and implicate clinical usefulness of IK channel openers.

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REFERENCES

Ahluwalia J, Tinker A, Clapp LH, Duchen MR, Abramov AY, Pope S, Nobles M, Segal AW. The large-conductance Ca^{2+}-activated K^+ channel is essential for innate immunity. *Nature* 427: 853-858, 2004

Cao Y, Dreixler JC, Roizen JD, Roberts MT, Houamed KM. Modulation of recombinant small-conductance Ca (2+)-activated K (+) channels by the muscle relaxant chlorzoxazone and structurally related compounds. *J Pharmacol Exp Ther* 296: 683-689, 2001

Chandy KG, DeCoursey TE, Cahalan MD, McLaughlin C, Gupta S. Voltage-gated potassium channels are required for human T lymphocyte activation. *J Exp Med* 160: 369-385, 1984

Estacion M. Characterization of ion channels seen in subconfluent human dermal fibroblasts. *J Physiol* 436: 579-601, 1991a

Estacion M. Acute electrophysiological response s of bradykinin-stimulated human fibroblasts. *J Physiol* 436: 603-620, 1991b

Ghanshani S, Wulff K, Miller MJ, Rohm H, Neben A, Gutman GA, Cahalan MD, Chandy KG. Up-regulation of the IKCa1 potassium channel during T-cell activation. Molecular mechanism and functional consequences. *J Biol Chem* 275: 37137-37149, 2000

Goodwin PA, Campbell KE, Evans BAJ, Wann KT. In vitro patch-clamp studies in skin fibroblasts. *J Pharma & Toxiclo Meth* 39: 229-233, 1998

Grgic I, Eichler I, Heinau P, Si H, Brakemeier S, Hoyer J, Kohler R. Selective blockade of the intermediate-conductance Ca^{2+}-activated K^+ channel suppresses proliferation of microvascular and macrovascular endothelial cells and angiogenesis in vivo. *Arterioscler Thromb Vasc Biol* 704-709, 2005

Jensen BS, Strobaek D, Olesen SP, Christophersen P. The Ca^{2+}-activated K^+ channel of intermediate conductance: a molecular target for novel treatments? *Curr Drug Targets* 2: 401-422, 2001

Jager H, Dreker T, Buck A, Gress T, Grissmer S. Blockage of intermediate-conductance Ca(2+)-activated K(+) channels inhibit human pancreatic cancer cell growth in vitro. *Mol Pharmacol* 65: 630-638, 2004

Koegel H, Alzheimer C. Expression and biological significance of Ca^{2+}-activated ion channels in human keratinocytes. *FASEB J* 15: 145-154, 2001

Kohler R, Wulff H, Eichler I, Kneifel M, Neumann D, Knorr A, Grgic I, Kampfe D, Si H, Wibawa J, Real R, Borner K, Brake meier S, Orzechowski HD, Reusch HP, Paul M, Chandy KG, Hoyer J. Blockade of the intermediate-conductance calcium- activated potassium channel as a new therapeutic strategy for restenosis. *Circulation* 108: 1119-1125, 2003

Lang F, Ritter M, Gamper N, Huber S, Fillon S, Tanneur V, Lepple-Wienhues A, Szabo I, Gulbins E. Cell volume in the regulation of cell proliferation and apoptotic cell death. *Cell Physiol Biochem* 10: 417-428, 2000

Lepple-Wienhues A, Berweck S, Bohmig M, Leo CP, Meyling B, Garbe C, Wiederholt M. K^+ channels and the intracellular calcium signal in human melanoma cell proliferation. *J Membr Bopl* 151: 149-157, 1996

Lim I, Yun J, Kim S, Lee C, Seo S, Kim T, Bang H. Nitric oxide stimulates a large-conductance Ca-activated K(+) channel in human skin fibroblasts through protein kinase G pathway. *Skin Pharmacol Physiol* 18: 279-287, 2005

Luo JD, Chen AF. Nitric oxide: a newly discovered function on wound healing. *Acta Pharmacologica Sinica* 26: 259-264, 2005

Manaves V, Zin W, Bauer AL, Rossie S, Kobayashi M, Rane SG. Calcium and vitamin D increase mRNA levels for the growth control hIK1 channel in human epidermal keratinocytes but functional channels are not observed. *BMC Dermatol* 16: 4-7, 2004

Nilius B, Wohlrab W. Potassium channels and regulation of proliferation of human melanoma cells. *J Physiol* 445: 537-548, 1992

Ouadid-Ahidouch H, Roudbaraki M, Delcourt P, Ahidouch H, Joury N, Prevarskaya N. Functional and molecular identification of intermediate-conductance Ca(2+)-activated K(+) channels in breast cancer cell: *Am J Physiol Cell Physiol* 287: C125-C134, 2004

Pardo LA. Voltage-gated potassium channel in cell proliferation. *Physiology (Bethesda)* 19: 285-292, 2004

Parihar AS, Coghlan MJ, Gopalakrishnan M, Shieh CC. Effects of intermediate-conductance Ca^{2+}-activated K^+ channel modulators on human prostate cancer cell proliferation. *Eur J Pharmacol* 471: 157-164, 2003

Pena TL, Rane SG. The fibroblast intermediate conductance K(Ca) channel, FIK, as a prototype for the cell growth regulatory function of the IK channel family. *J Membr Biol* 172: 249-257, 1999

Rouzaire-Dubois B, Milandri JB, Bostel S, Dubois JM. Control of cell proliferation by cell volume alterations in rat C6 glioma cells. *Pflugers Arch-Eur J Physiol* 440: 881-888, 2000

Wiecha J, Munz B, Wu Y, Noll T, Tillmanns H, Waldecker B. Blockade of Ca^{2+}-activated K^+ channels inhibits proliferation of human endothelial cells induced by basic fibroblast growth factor. *J Vas Res* 35: 363-371, 1998

Wonderlin WF and Strobl JS, Potassium channels, proliferation and G1 progression. *J Membr Biol* 154: 91-107, 1996