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Ion Channel Phenotype of Melanoma Cell Lines

D.H. Allen, A. Lepple-Wienhues, M.D. Cahalan
Department of Physiology and Biophysics, UC Irvine, Irvine CA 92717

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Abstract. Melanoma cells are transformed melanocytes of neural crest origin. K^+ channel blockers have been reported to inhibit melanoma cell proliferation. We used whole-cell recording to characterize ion channels in four different human melanoma cell lines (C8161, C832C, C8146, and SK28). Protocols were used to identify voltage-gated (K_V), Ca^{2+}-activated (K_{Ca}), and inwardly rectifying (K_{IR}) K^+ channels; swelling-sensitive Cl^- channels (Cl_{swell}); voltage-gated Ca^{2+} channels (Ca_V) and Ca^{2+} channels activated by depletion of intracellular Ca^{2+} stores (CRAC); and voltage-gated Na^+ channels (Na_V). The presence of Ca^{2+} channels activated by intracellular store depletion was further tested using thapsigargin to elicit a rise in $[Ca^{2+}]_i$. The expression of K^+ channels varied widely between different cell lines and was also influenced by culture conditions. K_{IR} channels were found in all cell lines, but with varying abundance. Whole-cell conductance levels for K_{IR} differed between C8161 (100 pS/pF) and SK28 (360 pS/pF). K_{Ca} channels in C8161 cells were blocked by 10 nM apamin, but were unaffected by charybdotoxin (CTX). K_{Ca} channels in C8146 and SK28 cells were sensitive to CTX (K_d = 4 nM), but were unaffected by apamin. K_V channels, found only in C8146 cells, activated at ~−20 mV and showed use dependence. All melanoma lines tested expressed CRAC channels and a novel Cl_{swell} channel. Cl_{swell} current developed at 30 pS/sec when the cells were bathed in 80% Ringer solution, and was strongly outwardly rectifying (4:1 in symmetrical Cl^-). We conclude that different melanoma cell lines express a diversity of ion channel types.

Key words: Melanoma — Potassium channel — Chloride channel — Calcium channel — Sodium channel

Correspondence to: M.D. Cahalan

Introduction

Melanoma incidence has risen 2–3-fold in the last 35 years (Evans & Manson, 1994), one of the fastest rates of any form of cancer. In melanoma, melanocytes transform into cells which are both rapidly proliferative and highly metastatic. Melanoma responds poorly to chemotherapy and radiotherapy; several alternative methods of treatment have been investigated, including hormone therapy (Rizk & Ryan, 1994) and immunotherapy (Evans & Manson, 1994).

A large body of evidence has been generated in lymphocytes concerning the role of ion channels in cellular mitogenesis (for review, *see* Lewis and Cahalan, 1995). High-affinity peptide blockers of voltage-activated K^+ (K_V) channels inhibit proliferation. In addition, lymphocyte mitogens stimulate an increase in the number of K^+ channels expressed by individual cells. K_V channels expressed by lymphocytes, although few in number (tens to hundreds per cell), maintain a resting potential of about −50 mV. This negative resting potential augments the electrochemical gradient, which allows Ca^{2+} ions to move into the cell through channels in the plasma membrane. If no compensatory mechanism were present, inward Ca^{2+} flux would depolarize the cell membrane, decreasing the driving force for Ca^{2+} into the cell. K_V and calcium-activated K^+ (K_{Ca}) channels in the membrane counter this depolarizing effect. As $[Ca^{2+}]_i$ rises, K_{Ca} channels open and hyperpolarize the cell membrane, increasing the electrical gradient driving Ca^{2+} influx. Blocking K_V channels depolarizes the cell membrane, decreasing the driving force for Ca^{2+} entry. The elevated $[Ca^{2+}]_i$ generated by Ca^{2+} influx is required for the cells' ability to proliferate.

Although the connection between K^+ channels in the cell membrane and mitosis is well established in lymphocytes, it has yet to be examined in great detail for other cell lines. There is evidence that blocking K^+ channels inhibits proliferation in several different cell

Table 1. Component salt concentrations (mM) of internal solutions

Solution name	$[Ca^{2+}]_i$	$CaCl_2$	HEPES	Na_2ATP	Sucrose
K^+ aspartate (low Ca^{2+})	10 nM	1	10		
K^+ $MeSO_4$ (low Ca^{2+})	10 nM	1	10		
K^+ aspartate (high Ca^{2+})	1 μM	8.7	10		
K^+ $MeSO_4$ (high Ca^{2+})	1 μM	8.7	10		
Cs^+ glutamate	10 nM	1	10	4	100
Cs^+ Cl	10 nM	1	10	4	100
Cs^+ aspartate	10 nM	1	5		

All solutions contained 10 mM EGTA

types, including Schwann cells (Chiu & Wilson, 1989), neuroblastoma (Rouzaire-Dubois, Gerard & Dubois, 1993), brown fat cells (Pappone & Ortiz-Miranda, 1993) and melanoma (Nilius & Wohlrab, 1992; Lepple-Wienhues et al., 1993). Work done by Lepple-Wienhues et al. (1996) indicates a connection between K^+ channels and Ca^{2+} influx, similar to what has been described in lymphocytes. Further similarities between the mechanisms in lymphocytes and in melanoma cell lines remain to be determined.

As melanoma is typically resistant to traditional forms of chemotherapy and radiotherapy, other methods will be necessary to inhibit progression of this form of cancer. Attempts, using K^+ channel blockers, to inhibit proliferation in melanoma to date have employed tetraethylammonium (TEA), a rather low affinity selective blocker (Nilius & Wohlrab, 1992). Examination of previous work on ion channels in different melanoma cell lines, reveals a lack of consistent channel expression (Nilius, Bohm & Wohlrab, 1990; Lepple-Wienhues et al., 1993). The purpose of this study was to survey the diversity of channels present in melanoma. We describe the ion channel ''phenotype'' revealed by the patch-clamp technique in four different melanoma cell lines. Using protocols designed to isolate and distinguish distinct current components, eight channel types are described, including a novel Cl^- channel activated by cell swelling. Portions of this work have appeared previously in abstract form (Allen, Lepple-Wienhues & Cahalan, 1995).

Materials and Methods

Cell Culture

C8161, C832C, and C8146 melanoma cells were isolated and established as clonal lines in Dr. Frank Meyskens' laboratory (Department of Medicine, UCI); (Yamanishi & Meyskens, 1995). Biopsied samples were taken from patients with malignant melanoma. Single-cell suspensions were obtained, and plated for culture in soft agar. Spherical colonies of cells floated free of the agar, were collected, and dispersed into a single-cell suspension for final culturing. SK28 cells were obtained from ATCC. C8161, C832C, and C8146 cells were maintained in Ham's F-10 + 10% fetal bovine serum (FBS) (Summit Biotech, Ft. Collins, CO or Gemini Bioproducts, Calabasas, CA). SK28 cells were maintained in RPMI 1640 + 10% FBS (Summit). Unless specified, tissue culture medium contained FBS from Summit Biotech. All growth media were supplemented with 100 IU/ml penicillin and 100 μg/ml streptomycin and 25 mM N-(2-hydroxyethyl) piperazine-N′-(2-ethanesulfonic acid) (HEPES). Cells were maintained in a 95% air/5% CO_2 atmosphere at 37°C.

Solutions

The components (in mM) of pipette solutions containing K^+ or Cs^+ as the primary cations are summarized in Table 1. K^+ aspartate and K^+ $MeSO_4$ solutions were prepared with final calcium concentrations of 10^{-8} and 10^{-6} M, buffered with ethylene glycol-bis-(β-aminoethyl ether) N, N, N′, N′-tetraacetic acid (EGTA). Cs glutamate and CsCl solutions were supplemented with 100 mM sucrose, to create an osmotic gradient across the cell membrane, and 4 mM ATP. All solutions contained K^+ or Cs^+ salts at a final concentration of 160 mM, 2 mM $MgCl_2$ and 10 mM EGTA. The osmolarity of all internal solutions, except those used to study the swelling-activated Cl^- channel, was approximately 290 mosm.

Cells were bathed in Ringer solution consisting of (mM): 160 NaCl, 4.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, and 5 HEPES, titrated to pH 7.4 with NaOH. All anion- and cation-substituted Ringer solutions were made by replacing NaCl with the appropriate salt. Propionate and gluconate Ringer solutions were made with 160 mM acid, titrated with NaOH. Voltage-dependent block of the inward rectifying K^+ channel (K_{IR}) was investigated using a solution consisting of (mM): 150 KCl, 10 CsCl, 2 $CaCl_2$, 1 $MgCl_2$, and 5 HEPES, titrated to pH 7.4 with KOH. The Ba^{2+} aspartate solution contained 105 mM $BaOH_2$, titrated to pH 7.0 with aspartic acid. 5 mM HEPES was then added and the solution was further titrated to pH 7.4 with $BaOH_2$. Ba^{2+} Ringer contained 105 mM $BaCl_2$ and 5 mM HEPES. The osmolarity of all external solutions was approximately 305 mosm.

Patch-Clamp Techniques

Experiments were performed on cells which had been plated between 0.5 and 4 days previously. Standard whole cell recording techniques were applied (Hamill et al., 1981). Recording pipettes were pulled from soft glass capillaries (Accu-fill 90 Micropets; Becton, Dickinson, Parsippany, NJ), coated with Sylgard (Dow Corning, Midland, MI) near the tips, and fire polished to give resistances of 1–4 MΩ when filled with internal solution and immersed in Ringer solution. All membrane voltages were corrected for junction potentials between the pipette and bath solutions. The pipette current was zeroed before seal

formation. Cell membrane and pipette capacitance were canceled using electronic feedback via the patch clamp amplifier (List L/M-EPC-7, Medical Systems, Greenvale, NY, EPC-9, HEKA, Lambrecht, Germany, or Axopatch-1B, Axon Instruments, Foster City, CA). A Scientific Micro Systems MDX or SMS 1000 computer, running the SAP/ANAL family of programs, generated voltage commands and digitized and analyzed the patch clamp current output for the EPC-7 and the Axopatch-1B. An Apple Macintosh Quadra 700 computer, running Pulse (HEKA, Lambrecht, Germany) generated voltage commands and analyzed output for the EPC9. Unless otherwise noted, the traces were not corrected for leak current. Experiments were conducted at room temperature (21–25°C). Summaries are presented as mean ± SD.

Calcium Imaging

Cells were grown on coverslips and incubated for 60 min at 37°C in culture medium containing 5 μM of the fura-2 derivative fura-PE_3-acetoxymethylester (Molecular Probes, Eugene, OR). This procedure resulted in an even distribution of fluorescent dye in melanoma cells. After mounting the coverslips on a microscope stage (Carl Zeiss, Oberkochen, Germany) at room temperature, the cells were rinsed with Ringer solution in order to remove extracellular dye. The light beam of a xenon arc lamp (Carl Zeiss) passed through a computer-controlled filter wheel/shutter device (Lambda 10, Sutter Instruments, Foster, CA). Video images averaged from 16 frames were collected at 350 and 380 nm excitation wavelength every 10 sec. The images were digitized at 8-bit resolution, converted to ratios and stored on a hard disk using a video-image processor (Videoprobe, ETM-Systems, Irvine, CA). Intensities at zero and saturating $[Ca^{2+}]_i$ were obtained by subsequent application of Ca^{2+}-free Ringer containing ionomycin (10 μM)/EGTA (10 mM), followed by Ringer containing 2 mM Ca^{2+}. Ratios were converted to final $[Ca^{2+}]_i$ using the equation of Grynkiewicz et al. (1985), assuming a K_d for fura-2 inside the cell of 300 nM (Negulescu & Machen, 1990).

Results

Each of the cell lines studied expressed a unique pattern of channels. Channels shown also depended on culture conditions.

K^+ Channels

In previous patch-clamp studies, different melanoma cell lines have shown a variety of potassium channel types (Nilius et al., 1990; Lepple-Wienhues et al., 1992). Our results confirm these findings. In the four cell lines tested, one inward rectifying (K_{IR}), two types of calcium-activated (K_{Ca}), and one voltage-activated (K_V) channel were studied.

K_{IR}

K_{IR} channels were present in all four cell lines. A voltage ramp protocol showing whole-cell currents at negative potentials revealed a small inward conductance in cells dialyzed with a pipette solution containing K^+ aspartate or K^+ $MeSO_4$ ($[Ca^{2+}]_i$ 10^{-8} M) (Fig. 1). A sodium

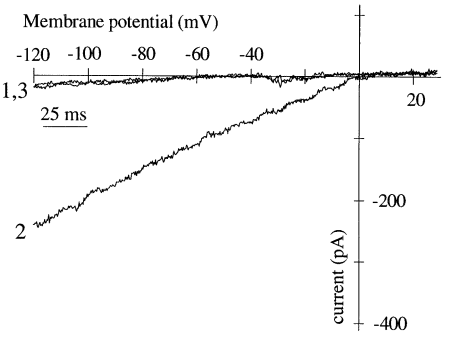

Fig. 1. Whole-cell currents elicited by 250-msec voltage ramps from −120 to 30 mV in C8161 cells. Traces 1 and 3: Ringer. Trace 2: K^+ Ringer. Whole cell conductance increases from 5 pS/pF to 67 pS/pF. Reversal potential changes from −60 to 0 mV. Pipette solution: K^+ aspartate (10^{-8} M Ca^{2+}).

current (discussed separately) appears as an inward current near −30 mV. Raising external $[K^+]$ to 160 mM shifted the reversal potential in the positive direction, and increased whole-cell conductance an average of fivefold ($n = 5$) (trace 2), further indicating that the current was carried by potassium. These channels appear to rectify weakly, as no negative slope conductance is seen in the voltage ramps. Ion permeability studies on the K_{IR} channel in C8161 cells were performed by replacing the Ringer solution with solutions containing various cations (Cs^+, TEA^+) substituted for Na^+. Neither of these ions significantly increased inward current. Substituting the 160 mM K^+ solution outside the cells with one containing 150 mM K^+ + 10 mM Cs^+ blocked the inward conductance in a voltage-dependent manner (*data not shown*). Overall, these experiments revealed the presence of a typical K_{IR} conductance in C8161, C8146, C832C and SK28 cells.

The K_{IR} channels in C8161 cells and SK28 cells showed different whole-cell conductance levels. With 160 mM K^+ outside the cell, conductance in C8161 cells averaged 101 ± 42 pS/pF ($n = 5$). Under the same conditions, SK28 cells had a whole cell conductance of 360 ± 122 pS/pF ($n = 16$). Cells within each line had a whole-cell capacitance of approximately 30 pF.

K_V

C8146 cells expressed a voltage-activated potassium channel. In voltage-ramp recordings conducted with a K^+ aspartate ($[Ca^{2+}]_i$ 10^{-8} M) pipette solution, an outward current activated at approximately −20 mV (Fig. 2*A*). Substitution of potassium for sodium outside the cell shifted the reversal potential to 0 mV, resulting in inward current from −20 to 0 mV, as predicted for a current carried by potassium. Whole cell conductance levels in normal Ringer solution, measured in three cells, aver-

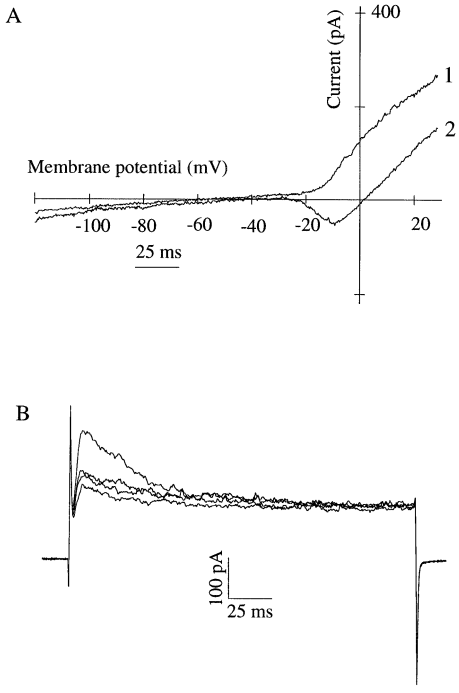

Fig. 2. Voltage-activated K^+ channels in C8146 cells. (*A*) Whole-cell currents elicited by 200-msec ramps from −120 to 30 mV. Trace 1: Ringer. Trace 2: K^+ Ringer. (*B*) Use dependence. Holding potential −80 mV. Voltage steps to 30 mV for 250 msec delivered at 1/sec. Pipette solution: K^+ aspartate (10^{-8} M Ca^{2+}).

aged 96 ± 14 pS/pF. The current inactivated with a τ_h of 22 ± 15 msec during voltage steps to 40 mV ($n = 3$). Repetitive pulsing at 1/sec led to a progressive decline in peak-current amplitude (use dependence) (Fig. 2*B*). These are characteristics similar to those seen in other voltage-activated potassium channels. C8161, SK28 and C832C cells showed no voltage-activated potassium channels when depolarized to positive potentials.

K_{Ca}

C8161, C8146 and SK28 cells both showed K_{Ca} channels, but with differing pharmacological sensitivities. When these cells were dialyzed with pipette solution containing K^+ aspartate or K^+ $MeSO_4$ ($[Ca^{2+}]_i$ 10^{-6} M), a calcium-activated current induced within 30 sec to a peak whole-cell conductance (measured between −20 and −40 mV) of 114 ± 32 pS/pF ($n = 11$) (Fig. 3*A*,*D*). When potassium was substituted for extracellular sodium, inward current increased (although a small part of this inward current is due to the activation of the K_{IR} channels by high external K^+), and the reversal potential shifted to 0 mV indicating that the primary ion carrying the current was K^+ (Fig. 3*B*). Cation-substitution experiments in C8161 cells showed a permeability ratio (K^+:Cs^+:Na^+) of 1:0.2:<0.01.

The K_{Ca} channels in the two cell lines demonstrated different drug sensitivity. K_{Ca} channels in C8161 cells were sensitive to apamin, but not charybdotoxin (CTX). 10 nM apamin blocked outward current 50–70% ($n = 3$) (Fig. 3*C*). K_{Ca} channels in SK28 and C8146 cells were sensitive to CTX at similar concentrations, but not apamin (Fig. 3*D*,*E*). C832C cells showed no increase in conductance levels when dialyzed with high internal calcium.

Cl^- Channels

Cl_{swell}

A swelling-activated chloride conductance was seen in all four cell types (Fig. 4). Cells were dialyzed with a Cs glutamate internal solution to block any inherent potassium conductances. Channel conductance (measured between 30 and 50 mV) developed at a rate of 30 pS/sec when the cells were dialyzed with hypertonic internal solution containing 100 mM sucrose. Replacement of external Cl^- by large anions, such as gluconate or aspartate, significantly reduced outward current, indicating that the current was carried by Cl^-. The channel exhibited strong outward rectification, with an outward to inward ratio of 4:1 at ±40 mV in symmetrical Cl^- solutions, was neither time- nor voltage-dependent, and was blocked 70% in a time-dependent manner by 100 μM DIDS. The channel did not show any voltage dependent inactivation under normal conditions, but did inactivate in the presence of 100 μM DIDS at positive voltages. Anion-substitution experiments showed a permeability ratio (Br^-:NO_3^-:I^-:Cl^-:gluconate:propionate) of 1.67:1.09:1.04:1:0.47:0.03. Channel properties and conductance magnitudes were indistinguishable in all cell types, and under all culture conditions.

Ca^{2+} Channels

Two types of Ca^{2+} channels were seen. A calcium-release-activated channel (CRAC) was present in all four cell types. SK28 and C8161 cells expressed a voltage-activated Ca^{2+} channel (Ca_V).

CRAC

A small inward current developed within 120 sec of establishing the whole-cell configuration, when an internal solution containing 10 mM EGTA and 10 μM 1,4,5 inositol trisphosphate (IP_3) was used to deplete intracellular stores (Fig. 5). The current was inwardly rectifying, reversed at positive voltages, and was completely blocked by extracellular La^{3+} (1 μM). This current was observed in three out of five C8161 cells.

To further ensure the presence of a calcium influx pathway activated by Ca^{2+} store depletion, intracellular

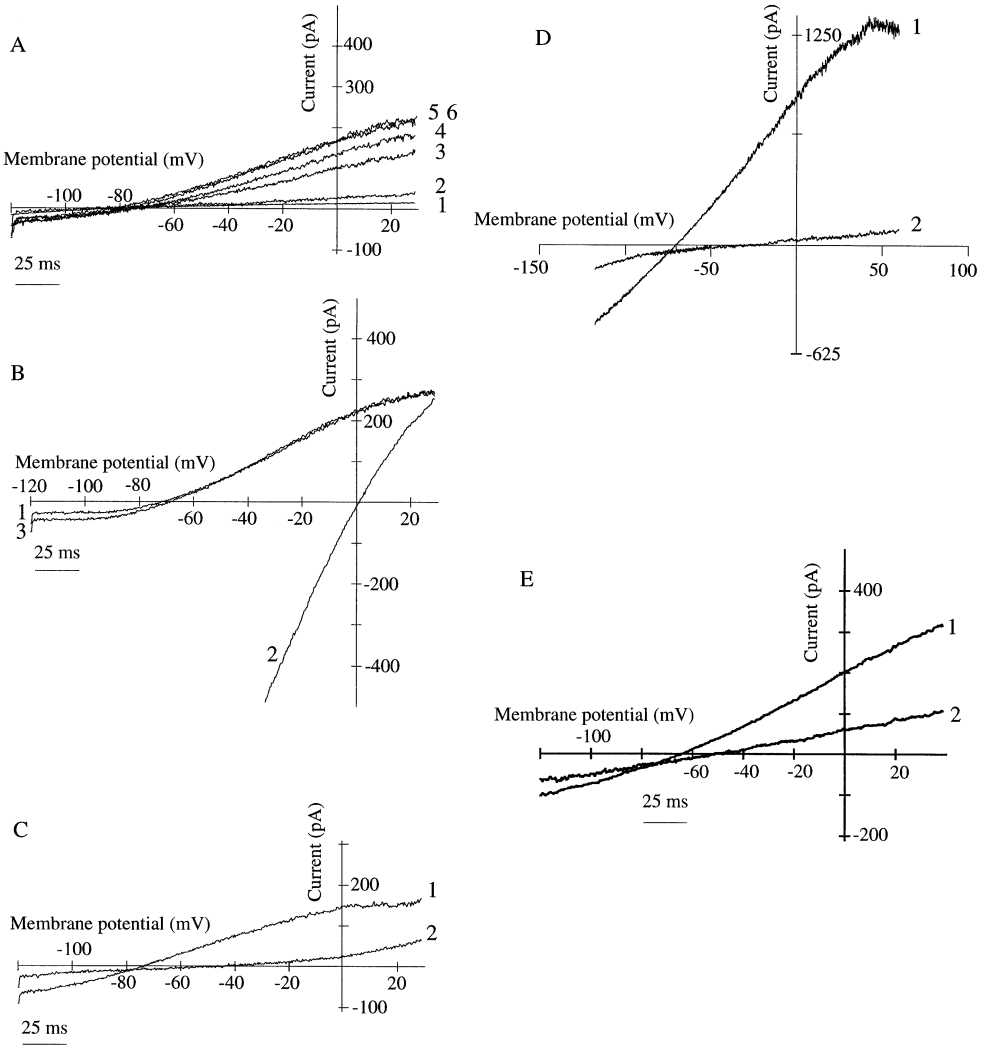

Fig. 3. Calcium-activated potassium channels in C8161 cells (*A–C*) and SK28 cells (*D*). Whole-cell currents elicited by 250-msec ramps from −120 to 30 mV. (*A*) Induction of K^+ current with 1 μM Ca^{2+} internal solution. The current induced quickly (within 30 sec) to a stable peak value of approximately 200 pA at 30 mV. (*B*) Current is carried by potassium. Traces 1 and 3: Ringer. Trace 2: K^+ Ringer. Inward current increased due to both increased flux through K_{Ca} channels and the opening of K_{IR} channels by high external K^+ levels. (*C*) Block of K_{Ca} in C8161 by apamin. Whole cell current was reduced 50–70% by the addition of 10 nM apamin outside the cells. Block of the current was irreversible. Pipette solution: K^+ aspartate (10^{-6} M Ca^{2+}). (*D*) Block of K_{Ca} channels in SK28 cells by charybdotoxin (CTX). Whole-cell current (trace 1), induced by 10^{-6} *M* Ca^{2+} in the pipette, was virtually eliminated by the addition of 10 nM CTX outside the cells (trace 2). The current block was completely reversible. (*E*) Block of K_{Ca} channels in C8146 cells by CTX. Whole cell current (trace 1), induced by 10^{-6} M Ca^{2+} in the pipette, was eliminated by addition of 100 nM CTX outside the cell (trace 2).

free Ca^{2+} was measured using fura-2 fluorescence. Thapsigargin (TG, 1 μM), added in the nominal absence of extracellular Ca^{2+}, depletes intracellular Ca^{2+} stores due to its inhibition of the intracellular Ca^{2+}−ATPase. Leakage from internal stores elevated $[Ca^{2+}]_i$ transiently (Fig. 5*B*). Subsequent readdition of external Ca^{2+} enabled Ca^{2+} influx, raising $[Ca^{2+}]_i$ to a peak which slowly declined as Ca^{2+} was pumped out of the cell. Depolarizing the membrane with elevated external $[K^+]$ (160 mM) or the addition of external La^{3+} (1 μM) inhibited the Ca^{2+} influx (*data not shown*). TG-activated Ca^{2+} influx was observed in 20 to 50% of cells, in all four melanoma lines.

Ca_V

Voltage steps, from a holding potential of −80 to −60 mV, activated a small inward current in two cell lines (C8161, SK28). Removal of external Ca^{2+} abolished the current; replacement of extracellular cations with Ba^{2+} increased the current. When stepped to −40 mV from the −80 mV holding potential, channels opened and then

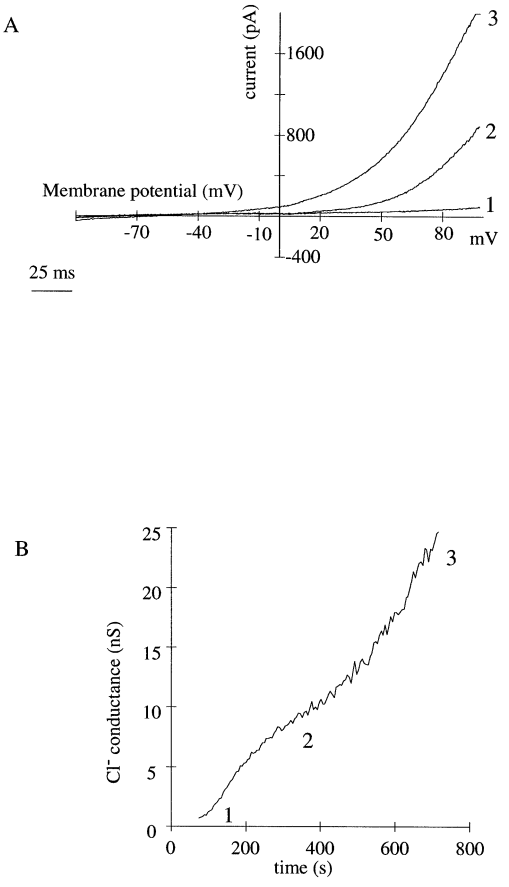

Fig. 4. Swelling induced Cl^- conductance in C8161 cells. (*A*) Whole cell currents elicited by 250-msec ramps from −100 to 100 mV at times indicated by corresponding numbers in *B*. (*B*) Slope Cl^- conductance plotted against time after seal formation. Cl^- conductance was determined from a linear least-squares fit to the current-voltage relation at potentials between 20 and 50 mV. Pipette solution: Cs glutamate + ATP + 100 mM sucrose. External solution: Ringer.

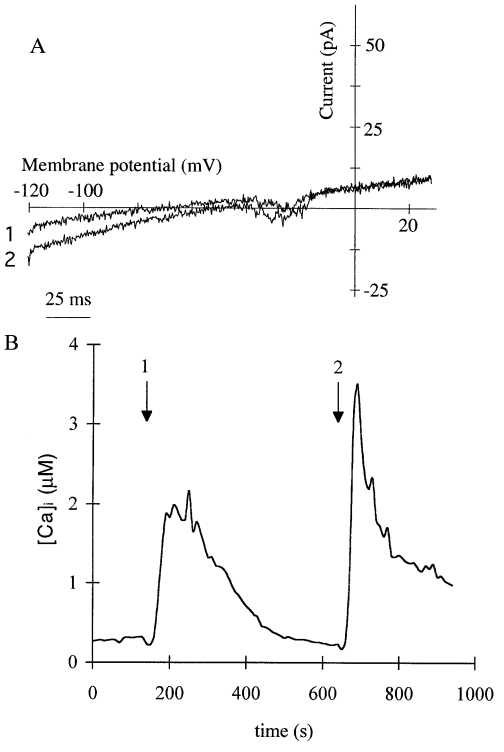

Fig. 5. Calcium currents in C8161 cells. Whole cell currents elicited by 250-msec ramps from −120 to 30 mV. (*A*) Induction of I_{CRAC}. Intracellular calcium pools were depleted using 10 mM IP_3 in the pipette solution. Traces 1 and 2 were recorded before and after activation of I_{CRAC}. (*B*) $[Ca^{2+}]_i$ measured with fura-2PE in SK28 cell. Average of 31 cells. Addition of 1 μM thapsigargin simultaneous with removal of external Ca^{2+} (arrow 1) resulted in a transient rise in $[Ca^{2+}]_i$. Readdition of 2 mM Ca^{2+} (arrow 2) resulted in Ca^{2+} influx.

inactivated rapidly with a τ of less than 10 msec. These properties are typical of a transient, low voltage-activated Ca^{2+} channel. With 10 mM Ba^{2+} as the charge carrier, the current was insensitive to La^{3+} (10^{-4} M), nifedipine (1 μM), and verapamil μM). This current will be described in more detail in a separate publication.

Na^+ Channels

Na_V

A voltage-activated Na^+ channel was seen in C8161 and C8146 cells. This channel appeared in approximately 40% of the cells tested. Voltage ramp protocols were routinely used to determine whether a particular cell expressed the channel. If any inward currents were seen, a family of depolarizations was delivered. Inward current peaked between −10 and 0 mV (Fig. 6*A*). Exchanging the Ringer solution with K^+ Ringer, NMDG Ringer, or Ba^{2+} aspartate eliminated the inward current, indicating that it was largely carried by sodium. The channel was blocked by TTX with a K_d of 6 nM (Fig. 6*B*). These characteristics are typical of voltage-activated Na^+ channels. SK28 and C832C cells did not show any inward conductances when the family of depolarizations was delivered.

Discussion

A large diversity of channel types are present in melanoma cells. Channel expression depended on the cell line and on culture conditions. The basic channel makeup of each of the cell lines we surveyed is presented in Table 2. Initial reports by Nilius (1990) and Lepple-Wienhues (1993) described a variety of channel types present in two different melanoma cell lines. Our results expand on these initial findings, through a more extensive survey of channel types and the addition of three new cell lines.

One pattern of channel expression emerges when cells from all experiments are observed. C8161, SK28

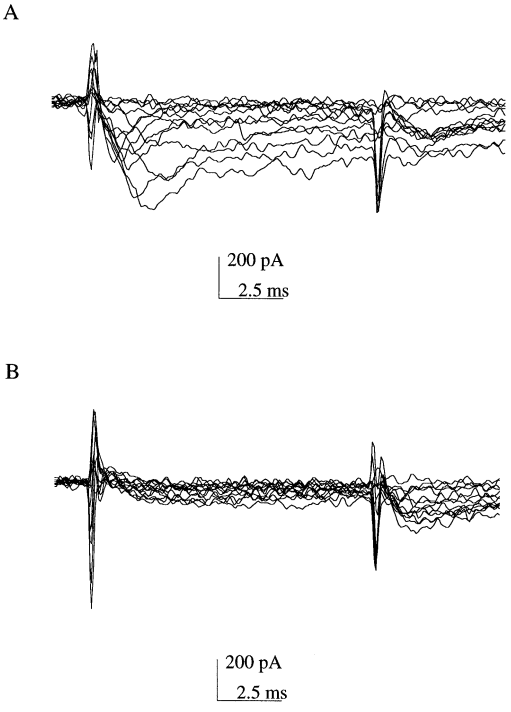

Fig. 6. Sodium currents in C8161 cells. Traces corrected for leak current. (*A*) Whole cell currents elicited by 20-msec voltage steps from −60 to 60 mV from a holding potential of −80 mV. (*B*) Inward current was blocked by the addition of TTX (10^{-6} M). Pipette solution: K^+ asparate (10^{-8} M Ca^{2+}).

and C8146 cells all expressed a K_{Ca} with a K_{IR}. Whole cell conductances of K_{IR} were different in the three lines, although all three cell types expressed a weakly inwardly-rectifying channel. The pharmacological sensitivities of the K_{Ca} varied, however. K_{Ca} channels in SK28 and C8146 cells were sensitive to CTX, and the K_{Ca} in C8161 cells were apamin-sensitive. C832C cells differ in that they possess a K_{IR} channel, but lack the K_{Ca}.

Blocking K^+ channels has been proposed as a possible method for inhibiting proliferation in many cell types, including melanoma cells. TEA and cyclic AMP inhibit cell proliferation in IGR1 melanoma (Nilius & Wohlrab, 1992). These compounds were selected based on their ability to inhibit the delayed rectifier type K^+ channels seen in IGR1 melanoma cells. In SK28 cells, a variety of IRK blockers inhibits proliferation (Lepple-Wienhues et al., 1996).

The role that K^+ channels play in cell division is not clearly understood. K^+ channels maintain the membrane potential at negative values, providing a large driving force for calcium entry into the cells. When Ca^{2+} channels in the membrane open, ions flow into the cell. The higher calcium levels inside the cell open K_{Ca} channels, further polarizing the membrane, increasing the driving force for calcium. To test whether the opening of K_{Ca} channels is necessary for cell division in melanoma, we examined the effects of a specific K_{Ca} channel blocker, apamin, on the ability for C8161 cells to incorporate [^{3}H] thymidine. Apamin had no effect on the cells' ability to uptake thymidine (*data not shown*). TEA used as a positive control in these experiments did inhibit uptake of thymidine. This result agrees with similar experimental results seen using CTX on SK28 cells (Lepple-Wienhues et al., 1993). The K_{Ca} channel does not appear important for allowing Ca^{2+} entry into the resting cell to levels necessary for cell division. It is important to note that under some conditions, C832C cells have no observable K^+ channels, yet they grow and divide normally.

Ca^{2+} can enter melanoma cells through two types of membrane channels. All four cell lines tested expressed a Ca^{2+} conductance activated by depleting internal calcium stores. This conductance displayed characteristics typical of the I_{CRAC} Ca^{2+} channels (Hoth & Penner, 1993, Lewis & Cahalan, 1989). Although all cells tested for I_{CRAC} by dialyzing IP_3 into the cells through a patch pipette activated the current, only approximately 50% of cells in imaging experiments did. This difference in activation probability indicates that the channel expression may be cell cycle linked.

The coexpression of a K_{IR} channel and CRAC in all four cell types is consistent with a Ca^{2+} signal being involved in melanoma cell proliferation. The K_{IR} channel seems to act as the conductance responsible for maintaining the membrane potential in melanoma cell lines.

Two of the cell lines, C8161 and SK28 cells, also possessed a voltage-activated Ca^{2+} channel. The negative activation voltages and fast inactivation of this channel indicate that it is a member of the large, relatively poorly classified member of low voltage-activated Ca^{2+} channels (Hille, 1992).

All cell lines also expressed a Cl^- channel activated by cell swelling. The Cl_{swell} conductance, although similar in activation to that reported in lymphocytes (Lewis, Ross & Cahalan, 1993), differed in two important ways. The permeability ratio ($I^- > NO_3^- > Br^- > Cl^-$ > propionate > gluconate in lymphocytes *vs.* $Br^- > NO_3^- > I^- > Cl^-$ > gluconate > propionate in melanoma) and the rectification ratio for the two conductances differed significantly (1.4:1 in thymocytes *vs.* 4:1 in melanoma, when measured at ±40 mV). These differences lead us to believe that melanoma cells may possess a member of the Cl_{swell} family of channels different from that reported in lymphocytes and a variety of other cell types (Lewis et al., 1993). The Cl_{swell} channel may be providing a driving force for Ca^{2+} entry in cells which do not express K_{IR} channels.

The types of channel present varied widely from one cell line to another, and according to culture conditions. Cell lines were initially grown in serum obtained from Summit Biotech. To more closely match the culture en-

Table 2. Channel expression in melanoma cells

Cell type	K_V	K_{Ca} (ap)	K_{Ca} (CTX)	K_{IR}	Cl_{SW}	Ca_{CR}	Ca_V	Na_V
C8161 (n = 94)	−	+(−)	−	+	+	+	+	+/−(−)
C8146 (n = 35)	+(−)	−	−(+)	−(+)	+	+	ni	−(+)
C832C (n = 10)	−	−	−	−(+)	+	+	ni	−
SK28 (n = 128)	−	−	+	+	+	+	+	−

Summary of four melanoma cell types (total number of cells studied) and the channels contained in each type. Presence of a particular channel within a line is indicated by a ''+'', absence by a ''−.'' Examples of cell types where Gemini serum yielded a different channel phenotype are enclosed in parentheses. In the case of the Na_V channel, the appearance in C8161 cells was near 40%. K_{Ca} (ap) = apamin sensitive K_{Ca} channel. K_{Ca} (CTX) = charybdotoxin sensitive K_{Ca} channel. ni = not investigated.

vironment in which the cell lines were initially characterized (Yamanishi & Meyskens, 1995), medium and serum were changed. When the cells were exposed to new culture conditions, channel phenotype changed. Often, cells within a line did not consistently express a particular channel. An example can be seen in the C8161 cells with the Na_V channel, which only 40% of cells express. C832C cells did not appear to express any K^+ channels at all when grown in Summit serum. The membrane potential for these cells was near 0 mV according to the reversal potential of the voltage ramps, consistent with this observation.

C8146 cells grown in Summit serum expressed a channel phenotype most closely related to the IGR1 melanoma cells studied previously (Nilius & Wohlrab, 1992). C8146 is the only line which expressed a K_V type (delayed rectifier) channel. There was, however, no indication of a nonselective cation conductance such as that seen in IGR1 cells.

Melanoma cells are considered to be electrically nonexcitable (Nilius & Wohlrab, 1992), yet they possess several types of voltage-activated channels. The presence of these channels seems unusual in a nonexcitable cell. The large number of voltage-activated channels seen in melanoma cells may be attributable to the cells' neural crest lineage.

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