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Table of Contents

Cover.....	1
SF 298.....	2
Table of Contents.....	3
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	15
Reportable Outcomes.....	15
Conclusions.....	15
References.....	17
Appendices.....	N/A

INTRODUCTION

An important component of Schwann cell (SC) physiology, which has remained relatively unknown in NF1, is the electrophysiology of affected cells and the relationship of ion currents to development and maintenance of the NF1 SC phenotype. We are studying the relationship between a K channel current, functionally expressed in NF1 SC (Fieber 1998; this report), and SC proliferation. Ion channels are membrane proteins that mediate electrical communication between cells of the nervous system and are vital to nervous system function. This K channel is opened in response to depolarizations of the cell membrane, which could occur in myelinating SC via excitatory discharge of the SC-ensheathed axon. There is evidence that suggests that these K currents are directly related to the proliferative capacity of many cell types and have a relationship to well characterized second messenger pathways that play a role in the cell cycle during development (Konishi 1990) and the development of certain disease states. By studying the K currents of NF1 SC and their relationships to 1) growth factors found to be abnormally expressed in human NF1 SC, 2) known second messengers that can be measured or manipulated, and 3) proliferation, and by making relevant comparisons to these features in normal SC, we hope to provide insights about the tumorigenic process in NF1. The purpose of this study is to examine the inter-relationships between K channels, ras, and neurofibromin in SC proliferation.

BODY

The scientific goals of the second year of this study encompass tasks from Technical Objectives 1, 2 and 3 outlined in the Statement of Work. These objectives as they were pursued this year can be summarized generally as pharmacological block of tumored K channels in primary cultures of neurofibroma- and neurofibrosarcoma-derived SC to demonstrate that block of K channels inhibits proliferation. In addition, to evaluate the specificity on inhibition of proliferation of *tumored* K channel block, we pharmacologically blocked K currents of proliferating normal SC, which were not expected to be tumored K currents.

The initial testing of these experimental procedures was summarized in the annual report from year 1, in which the K currents of SC in 4 cell cultures were characterized and reported. It was apparent from those experiments that there was a continuum of K current characteristics between normal SC and SC from different types of neurofibromas, and that more cultures had to be examined. The experiments described here can be considered a fuller exploration and expansion of these scientific ideas to 10 different SC cultures.

Because of the intermittent availability of both tumored and normal Schwann cell cultures, and our requirement to accomplish many different scientific experiments on a culture while it is still in an early stage of passage, we routinely tried to work down through as many of the tasks in these technical objectives as are applicable to any given cell culture, rather than take each task one at a time and complete it using all the necessary cell cultures.

Table 1. Cell cultures used in the past year.

Source	Name	Mitogen status during experiments	Time in culture	Current Type	Doubling Time (hrs)
Normal nerve:	PW01	naïve	10 d	A type; or no current	Not Determined
	DM7	-GGF 6 d-2wks	few wks	A type; small sustained DR type; biphasic	87.4 ± 9.3* -GGF 2 wks
	DM8	-GGF 6 d	few wks	A type, small sustained; DR type, biphasic	Not Determined
	PW257	-GGF, -forskolin 6 d-few wks	few wks	A type, small sustained; DR type, biphasic	38.4 ± 4.75*
Neurofibrosarcoma:	DM5	none	few wks	large, sustained DR type; biphasic	52.4 ± 3.95*
	DM6	none	few wks	large, sustained DR type; biphasic	47.3 ± 2.77*
	T265	none	8 mos	large, sustained DR type	52.6 ± 0.65*
Cutaneous Neurofibroma:	DM1	none	few wks	A type, biphasic; no current	62.8
Plexiform neurofibroma:	DM3	none	few wks	small sustained DR type; biphasic	48.8 ± 4.54*
Normal nerve of NF1 patient:	DM10	-GGF 6 d-4 wks	few wks	A type, small sustained DR type; biphasic	72.0 -GGF >4 wks; 63.2 -GGF 2 wks

*Standard Error

Studies on NF1 cell cultures studied in tissue culture confirm the findings of Fieber (1998) in NF1 neurofibrosarcoma cell lines. The most conspicuous difference between normal and tumored cultured SC K currents was that tumor cells functionally expressed outward delayed rectifier (DR) K current (Table 1; Fig. 2), while quiescent, normal SC had only a small, transient, outward "A type" K current (A current; Table 1; Fig. 1).

Assays for cell proliferation and electrophysiology were done on early passages of normal and NF1 cell cultures, except for neurofibrosarcoma T265 where the passage number was high. All cell cultures were SC-enriched and S-100+, and can be expected to remain so for the first few passages. While we have classified these tumors in individual categories according to the histological type of neurofibroma, etc., it is important to recognize that because these are human tumors and not samples from inbred animals, there is some variability in the electrophysiological characteristics of SC derived from each of these sources (Wallace et al 2000; Muir et al 2001). The data are summarized as averages in specific, named cultures, although comparisons between cultures representing neurofibrosarcomas, for example, were made when possible. Current amplitudes are directly comparable even though the size of cells varies, because the currents (in picoamperes, pA) are reported by normalizing to the cell's capacitance (in picofarads, pF).

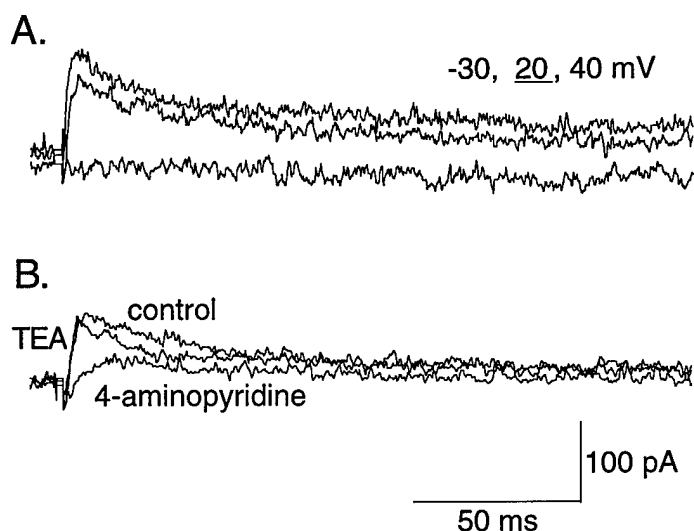


Fig. 1. K currents in normal SC plated on laminin but naïve to GGF or other exogenous growth factors. A. Family of K currents to the indicated test potentials. B. Pharmacological experiments at 20 mV to identify the K currents as inactivating, A type K currents preferentially blocked by 4-aminopyridine.

Currents

Quiescent normal SC (PW01) naïve to exogenous growth factors such as recombinant human heregulin (rh-heregulin; also known as glial growth factor (GGF)) had K currents with the same characteristics as those reported in Fieber 1998. These were small amplitude outward K currents of the transient, A type, blocked by 4-aminopyridine (Fig. 1A, B; Table 2). Inward rectifier K currents (IR), and Na⁺ currents were occasionally observed. These normal SC were studied soon after dissociation of the nerve (10 d), because SC cultured alone (for example, without neurons) require GGFs (GGF or epidermal growth factor (EGF) + forskolin) for long term survival in culture. The presence in whole cell recordings of IR K currents may be a function of the cells' age in culture or time removed from the nerve (Konishi 1994). In 10 PW01 cells, 4 had A type currents of 4.84 ± 0.54 pA/pF. The remaining 6 cells had no outward currents. Many normal SC do not have recorded currents (Fieber 1998).

We expected that placing our cryopreserved, normal SC stocks from the DM series enriched for SC with GGF (DM8, DM7), or GGF-expanded cultures (never cryopreserved) from the PW series (PW02, PW257) into normal medium without exogenous growth factors such as GGF or forskolin would result in quiescent cultures. 6 d after withdrawal of GGF, normal SC proliferated in culture. Doubling times ranged from a low in DM7 of 80.4 ± 8.8 hrs to a high in PW257 of 38.4 ± 4.75 hrs (PW257 was withdrawn from 1 mM forskolin at the same time it was withdrawn from GGF). Electrophysiological measurements were made in matched cultures (same cell passage as for proliferation assays). The pharmacological profile of the K currents of dividing normal SC demonstrated that the cells exhibited small, pure, A type currents, small outward DR type currents, or biphasic K currents composed of 4-AP- and tetraethylammonium- (TEA-) blocked components representing A type and DR type K currents, respectively (see Fig. 2B

for an example of a biphasic current in a tumored SC). IR K currents and Na⁺ currents were much rarer.

We plan to examine currents in normal SC with time after withdrawal of GGF to determine when GGF's effects disappear. This information will be important for studying relevant K currents in SC derived from the normal nerves of NF1 patients (see below).

Table 2. Characteristics of currents.

Current	Description	blocked by	Naive normal SC	Normal SC on GGF +/- forskolin	Normal SC, wks off GGF	Normal SC from NF1 patient	Cutaneous neurofibroma	Plexiform neurofibroma	Neurofibrosarcoma
A type	transient, decays with time	4-AP	++	++	++	+	++	+	rare
DR type	sustained, does not decay	TEA	-	small DR type in some	small DR type in some	small DR type in some	-	small or large DR type	++
biphasic	A+ DR	4-AP/TEA	-	+	+	+	+	+	rare
IR	inward K current	Barium, Cesium	+	rare	rare	rare	rare	rare	+
Na ⁺	inward Na ⁺ current	tetrodotoxin	+	+	-	-	-	rare	+

The SC of all *tumor* cultures we studied divided in normal culture medium without exogenous growth factors such as GGF or forskolin. The highest proliferation rates observed were in the neurofibrosarcoma cell lines T265, DM5 and DM6, with doubling times of ~50 hrs. To test the durability of these high proliferation rates over time, neurofibrosarcoma cultures were kept in culture for a minimum of 8 wks with no obvious senescence; T265 was kept in culture for 7 mos. DM3 was robustly dividing after 4 wks' study, with a doubling time of 48.8 ± 4.54 hrs. Only DM1 senesced under these conditions, at approximately 11 wks in culture, yet at 9 wks in culture DM1 was proliferating with a doubling time of 62.8 hrs.

The hallmark outward K current of all tumored SC was the tumored delayed rectifier (DR) K current blocked by TEA (Fig. 2). Transient, A type K currents and Na⁺ currents were occasionally seen, as were, rarely, IR currents (Table 2). The mean amplitude of DR K currents of different tumored SC cultures ranged 10-50 pA/pF, with neurofibrosarcomas, in general, having the largest DR currents.

K currents in all the *neurofibrosarcoma* cell lines (T265, DM5 and DM6) were classical tumored K currents (Fig. 2A). An interesting continuum of current profiles was seen in different *neurofibroma* cultures. The culture from DM3, a plexiform neurofibroma, had many cells characterized by classic tumored DR K currents, but some DM3 cells had biphasic currents that were also characteristic of proliferating normal cells (described

separately above). Biphasic K currents are composed of 4-AP- and TEA-blocked components representing A type and small DR type K currents, respectively (Fig. 2B). Yet all K currents in a cutaneous neurofibroma (DM1) resembled those of proliferating normal SC, with 3 of 10 cell studied having no current and 3 others having A type or biphasic outward K currents, with no cells exhibiting pure tumored DR current.

The variation of K current types in neurofibroma cultures raises the possibility that neurofibroma SC ion channels are not physiologically abnormal or that the ion channel phenotypes of normal, neurofibroma-derived and neurofibrosarcoma-derived SC represent a continuum that requires closer observation for differences between normal and neurofibroma-derived SC. Examination of more neurofibroma cultures is planned to explore this hypothesis.

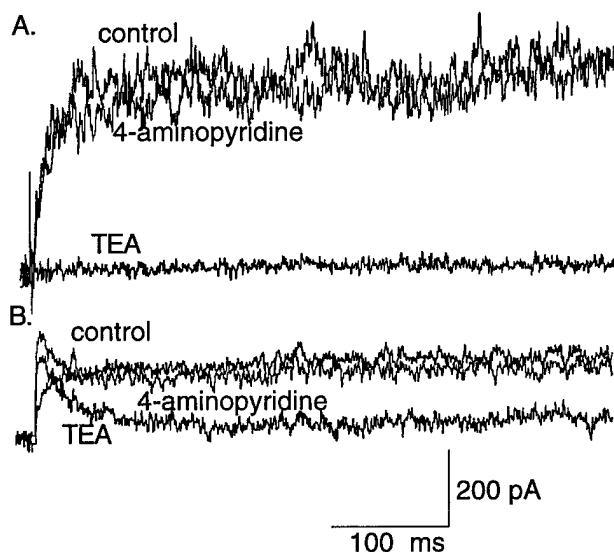


Fig. 2. K currents in tumored SC.

A. Pharmacological experiments at 20 mV in a T265 neurofibrosarcoma cell to identify the K currents as sustained, DR K currents, preferentially blocked by TEA. This cell has a small current component blocked also by 4-aminopyridine.

B. Pharmacological experiments at 20 mV in a DM3 plexiform neurofibroma cell to identify the biphasic K currents. This cell has a sustained, DR K currents component blocked only by TEA, and an inactivating A type component blocked only by 4-aminopyridine.

An important difference between the DR K currents of normal SC and those of tumored SC was that in normal dividing SC, the DR currents were smaller in amplitude than in any tumored cell culture. Mean K current amplitudes of all normal SC cultures PW257, DM7 and DM8 were significantly different than mean current amplitudes of all neurofibrosarcoma cell cultures DM6, DM5 and T265 (T-test; individual normal SC cultures vs. DM6: $p=0.0012$ for PW257; $p=0.0011$ for DM7; $p=0.0006$ for DM8. Normal vs. DM5: $p=0.0005$ for PW257; $p=0.0023$ for DM7; $p<0.0001$ for DM8. Normal vs. T265: $p<0.0001$ for all of PW257; DM7; DM8.). K current amplitudes in one normal culture, DM8, were significantly different from those in DM3, the plexiform neurofibroma ($p=0.0270$). K current amplitudes in DM1, the cutaneous neurofibroma were not significantly different from those in any normal SC culture.

DM10 is a very rare culture type, a SC culture derived from the normal nerve of an NF1 patient. Its neurofibromin status is unknown. This culture was SC-enriched with GGF before frozen stocks were made, but it has not been exposed to GGF in our lab. At the time of this writing, DM10 has been in culture in normal medium for 4 wks and its cells are still dividing, with a doubling time of 72 hrs (Table 1). K currents of DM10, recorded when the cells had been maintained in normal, GGF-deficient medium for 7-8 days, so far are characteristic of normal cells expanded with GGF described above, with 2 cells exhibiting A type currents, 2 cells exhibiting biphasic currents and 7 cells exhibiting tumored DR type currents.

Technical Objective 3 of the current grant was to compare the ion channel features of SC in cultures like DM10, from normal nerves of NF1 patients, with the other SC cultures already described, to assess the ionic currents of cells in which neurofibromin may be downregulated (normal-appearing NF1 SC). We have 1 other such culture (DM9) and will continue to grow them both without GGF to see if their proliferation stops (but cells survive quiescent), and if so, what the currents look like in quiescent SC from these cultures. It is possible that in culture, at least, removed from axonal controls, SC from normal nerves of NF1 patients such as DM10 continue to proliferate, providing an important intermediate category of SC to study in this model system. The basal level cAMP of DM10, its responsiveness to CPTcAMP, etc, will be a focus of immediate future studies.

TEA analogs block SC proliferation and SC K currents

Technical Objective 1 of the current grant period was to pharmacologically block tumored delayed rectifier (DR) K channels in tumored SC to demonstrate that K channel block inhibits proliferation. The classical blocker of DR K currents is tetraethylammonium (TEA). We used 2 analogs of TEA that do not appear to be toxic to cells: tetrapentylammonium (TPeA; 50 μ M) and tetrahexylammonium (THeA; 5 μ M; Wilson and Chiu, 1993); and exposed separate cultures to each of these analogs for 54 hrs before assaying for cell proliferation using the BrdU immunocytology assay (Zymed). In 3 neurofibrosarcoma cell lines (Table 3) and in 2 neurofibroma cell cultures optimized to contain only SC (Table 4), these agents completely or almost completely inhibited tumored SC proliferation. THeA and TPeA treatments were approximately equal in their effects, thus block is summarized as "% inhibition by TEA analogs". We verified that THeA and TPeA also blocked K currents in tumored cells at the same concentrations that blocked proliferation, either severely reducing or abolishing recorded whole cell K currents compared to matched controls (same cell passage; Table 3). The current block washed out within minutes after 24 hrs' exposure to TEA analogs, confirming that THeA and TPeA blocked currents by the conventional manner of a channel plug rather than by causing their downregulation. Cells exposed to TEA analogs for up to 48 hrs appeared healthy in all respects, with a membrane sufficiently intact to do electrophysiological experiments. After 72 hrs in 5 μ M THeA, membrane seals were more difficult to obtain.

The resting potential (RP) of a cell defines the difference in ion concentrations on either side of the plasma membrane. Its value is determined primarily by the difference in K^+ concentration on either side of the membrane, as maintained by the different routes for K^+ entry, such as ion channels, the Na^+/K^+ ATPase, and leak conductances. The control RPs of SC in different neurofibroma- and neurofibrosarcoma-derived SC cultures averaged -26 to -44 mV (T265, DM5, DM6, DM1 and DM3). TEA analogs applied for >40 hrs depolarized RPs by $>50\%$ of their control values (Table 3). The reduced RPs observed in TPeA and THeA were significantly different from controls (t-test; $p \leq 0.043$ for individual TPeA and THeA comparisons with their matched controls). Within 1 hr of washout of TEA analogs (and relief of DR K channels from block), RPs in THeA- and TPeA-exposed cells were not significantly different from matched controls.

We did one experiment to test the idea that the inhibition of proliferation was a secondary consequence of the change in RP caused by TEA analogs. We monitored proliferation and K currents in the cell line T265 after 54 hrs' exposure to 10 or 13 mM added KCl (added to the culture medium) to mimic the decrease in RP in these cells effected by the TEA analogs. This procedure for reducing RP should have little effect on voltage-gated K channels. Although added KCl reduced RP by the same amount as TEA analogs, it was without effect on proliferation rates or K current amplitude. We hypothesize that depolarization of the RP is not the primary cause of inhibition of proliferation by TEA analogs, but rather a consequence of K channel block. This idea is supported by the hypothesis of Kodala et al (2000) who speculated that when inward rectifier (IR) K channels are present at very low density (as seems to be the case in cultured SC in these experiments) the opening or closure of just a few DR K channels can cause fluctuations of the RP as large as tens of mV. The absence of IR depolarizes cells, which drives open DR K channels. DR channel opening hyperpolarizes the RP again. But if the DR channels are blocked, as by TEA analogs, then the RP remains depolarized.

Another experiment testing the role of RP independent from most effects on K channels is to depolarize RP via ouabain-induced inhibition of the Na^+/K^+ ATPase. If ouabain-induced depolarization inhibits proliferation, then depolarized RP may play a role in inhibition of proliferation, whereas if proliferation is not affected by ouabain, K channel block is probably more important. Ouabain-induced depolarization of proliferating rabbit SC in culture (Pappas and Ritchie 1998) had no effect on proliferation.

Table 3.
Effect of TEA analogs on cell proliferation and electrophysiological parameters in neurofibrosarcoma- and neurofibroma-derived SC.

	neurofibrosarcomas			neurofibromas	
	T265 (Nf1 ND)	DM5, (Nf1-)	DM6 (Nf1+)	DM1 (Nf1-)	DM3 (Nf1-)
% inhibition of cell prolif. by TEA analogs	$94 \pm 6\%$	$100 \pm .03\%$	$96.5 \pm 2.6\%$	$71.5 \pm 28\%$	$100 \pm 0\%$
Electrophys'1 summary of effects of	$>75\% \downarrow$ in I_K amplitude, RP 26 mV \downarrow	$>60\% \downarrow$ in I_K amplitude, RP 24 mV \downarrow	$>65\% \downarrow$ in I_K amplitude, RP 13 mV \downarrow	Not Determined (ND)	$>60\% \downarrow$ in I_K amplitude, RP 12 mV \downarrow

TEA analogs					
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Thus the most important conclusion of the study to date is that TEA analogs block tumored SC K channels and proliferation after 2 d exposures, without apparent damage, death or changes in morphology. This suggests that K channels have a role in SC proliferation.

Another aspect of Technical Objective 1 was to pharmacologically block K channels in normal, proliferating SC to evaluate the specificity of tumored DR K current block on inhibition of proliferation in human SC. An assumption going into these experiments, based on data from Fieber (1998), was that normal proliferating SC would not exhibit the tumored DR K current, and so TEA analogs would reveal clues about the presumed different mechanisms of proliferation in normal vs. tumored SC. Proliferation in normal SC cultures was blocked by TEA analogs (DM7, Table 4a), which may be related to the functional expression of some small amplitude DR K currents in proliferating normal SC cultures.

Proliferation of normal SC in GGF was not significantly different than proliferation of normal SC that had been removed from GGF for >2 wks (Table 4a), supporting our suspicion that the effects of GGF are long-lasting. Addition of the membrane permeant cAMP analog CPTcAMP (1 μ M) to normal PW257 cells withdrawn from GGF +1 μ M forskolin for 2 wks did not increase their proliferation rate significantly. Adding CPTcAMP to PW257 cells exposed continuously to GGF (but off forskolin for 2 wks) did not further increase proliferation. These results support the idea that CPTcAMP alone does not stimulate proliferation of normal SC, nor does it potentiate the effects of GGF, unlike forskolin (Kim et al 1997a).

RPs and the K current amplitudes and current types observed in normal SC cultures were not affected by CPTcAMP or GGF treatment compared to control cells from which GGF had been withdrawn for 2 wks prior to experiments (Table 4b).

The final part of Technical Objective 1 was to test the link between proliferation and elevated intracellular cAMP levels in tumored cells. We have begun to examine this using a colorimetric immunoassay kit for cAMP (Sigma). Although it is expected that cAMP levels may fluctuate in SC with the cell cycle stage (Kim et al 1997a), it is also possible that tumored or dividing SC have chronically elevated cAMP, which stimulates SC to divide in vitro (Mirsky and Jessen 1996).

Testing and interpretation of the results obtained with the Sigma immunoassay for measuring intracellular [cAMP] in cell cultures has required more time than expected. At this point, the assay seems to be most useful as a tool to compare qualitative shifts in [cAMP] under different experimental conditions (eg, control vs. TEA-analog-exposed cultures) rather than as a quantitative indicator of [cAMP]. The ¹⁻¹²⁵cAMP radioassay is an alternative assay for cAMP successfully used by some laboratories. We will consider its use if the immunoassay continues to be so modestly fruitful.

Table 4.
NORMAL SCHWANN CELLS
a. Proliferation experiments

PROLIFERATION							
Normal SC	TEA analogs*	FTI III 10 μ M*	FTI III 50 μ M*	No treatment*	25 ng/ml GGF for > 2 wks	0.5 μ M CPTcAMP*	GGF + CPTcAMP
DM7, Nf1+	100 \pm 0% inhib	87.5 \pm 0.9% inhib	100 \pm 0% inhib	87.4 \pm 9.3 hr doubling time	66.5 hr doubling time	ND	ND
PW257 Nf1 ND	Not Determined (ND)	86 \pm 0% inhib	ND	38.4 \pm 4.75 hr doubling time	50.9 \pm 10.3 hr doubling time	26% \pm 4.0% inc v. no treatment	3.0 \pm 1.0% inc v. +GGF alone
DM10** Nf1 ND	100 \pm 0% inhib	95 \pm 0% inhib	ND	72 hr doubling time	63.2 hrs doubling time	ND	ND

b. Electrophysiological experiments

ELECTROPHYSIOLOGY RESULTS							
Normal SC	TEA analogs	FTI III 10 μ M	FTI III 50 μ M	No treatment*	25 ng/ml GGF	1 μ M CPTcAMP	GGF + CPTcAMP
DM7	ND	ND	ND	-24 \pm 5.7 mV RP, small I_K amplitude	No change in RP, No change in I_K	ND	ND
PW257	ND	ND	ND	-25 \pm 9.8 mV RP, small I_K amplitude	No change in RP, No change in I_K	No change in RP, No change in I_K	No change in RP, No change in I_K
DM10**	ND	ND	ND	-22.2 \pm 9.02 mV RP, small I_K amp	ND	-31.3 \pm 4.93 mV RP, No change in I_K	ND

* note proliferation of normal cells after GGF withdrawal from DM7 for 6 d, from PW257 for 2 wks.

For P257, significance of differences in RP and I_K amp have been tested and all groups against one another in pairs are NSD.

** DM10 is a normal nerve-derived culture from an NF1 patient

Effects of blocking ras on proliferation and currents

Technical Objective 2 of the current grant period was to study how ras affects the functional expression of different K channel types by application of membrane permeant farnesyl transferase inhibitors (FTI) or antibody Y13-259 to block ras. Neurofibromin has been proposed to act as a tumor suppressor, thereby inactivating ras. Our hypothesis was as follows: If neurofibromin controls ras levels, reducing ras might mimic normal neurofibromin, returning neurofibroma-derived and neurofibrosarcoma-derived SC to a normal SC profile with respect to proliferation and K current type. (Yan et al, 1995; Kim et al, 1997a). This hypothesis assumes ras levels are elevated in NF1-derived SC.

All our data to date were collected using FTI to block ras. We applied FTI (FPT Inhibitor III, Calbiochem), two days before BrdU proliferation assays and/or electrophysiological experiments in tumored SC (T265, DM5, DM6, DM1 and DM3) and normal SC (DM7, PW257, and DM10, the normal nerve-derived culture from an NF1 patient). Controls were passage- and time-matched cultures. In all neurofibrosarcoma, neurofibroma and normal cultures tested, both 10 μ M and 50 μ M FTI inhibited proliferation (Tables 4a, 5 and 6).

FTI did not revert the tumor cells to a normal morphological phenotype, however. Several morphological changes were observed that featured an increased prominence of the cytoskeleton, but we have not completed the analysis of these features.

Electrophysiological measurements on tumor cells to date demonstrated that although a small depolarization of the RP occurred in FTI, the size of the K currents was not significantly different, nor were there obvious changes in channel phenotype (Table 5). An apparent FTI-induced current reduction in plexiform neurofibroma culture DM3 was not a statistically significant difference (Table 6). However, the size of DR K currents varies widely between individual SC in a culture, and more recordings from more cell lines are needed to confirm or refute the lack of effect of FTI on K current amplitude. It is possible, nevertheless, that K channel modulation by ras-activated pathways is sufficiently complex that blocking ras alone may not revert the electrophysiological characteristics of tumored cells to normal.

We have not yet tested if cAMP levels are altered by FTI.

Table 5.
Effect of farnesyl transferase inhibitor on cell proliferation and electrophysiological parameters in neurofibrosarcoma-derived SC

	neurofibrosarcomas					
	T265		DM5		DM6	
	10 μ M FTI	50 μ M FTI	10 μ M FTI	50 μ M FTI	10 μ M FTI	50 μ M FTI
% inhib cell prolif	2 \pm 0%	69 \pm 0%	58 \pm 0%	94 \pm 6%	64 \pm 19%	99 \pm 0.5%
Electrophys summary	No change in I_K amplitude, RP 17 mV \downarrow	No change in I_K amplitude, RP 31 mV \downarrow	No change in I_K amplitude, RP 4 mV \downarrow	No change in I_K amplitude, RP 10 mV \downarrow	Not determined	Not determined

Table 6.
Effect of farnesyl transferase inhibitor on cell proliferation and electrophysiological parameters in neurofibroma-derived SC

	neurofibromas			
	DM1		DM3	
	10 μ M FTI	50 μ M FTI	10 μ M FTI	50 μ M FTI
% inhib cell prolif	Not determined	Not determined	81 \pm 0%	100 \pm 0%
Electrophys summary	Not determined	Not determined	No change in I_K amplitude	No change in I_K amplitude

Characterizing K currents of dividing vs. resting cells

Finally, to begin to pinpoint which K currents are characteristic of tumored SC that are dividing vs. quiescent, we did electrophysiological experiments on cells then tested whether specifically these cells had been dividing at the time of the recordings. We recorded K currents from DM3 plexiform neurofibroma SC with lucifer yellow (1 mg/ml) in the patch pipette, then fixed the cultures and ran the proliferating cell nuclear antigen (PCNA) assay, indicative of cells that are in G2 at the time they were fixed (Zymed). Of 13 cells recorded, 9 were re-identified after PCNA staining via lucifer yellow fluorescence. The 7 PCNA negative (approximately non-dividing) SC had A type transient or biphasic K currents consisting of A current and a small DR K current. The 2 PCNA positive (soon to divide) SC had sustained DR type K currents. This technique, though laborious, has high potential for pinpointing changes in K currents that correlate with changes in cell cycle.

Due to the variability in K current phenotypes seen in individual cells from normal and neurofibroma- (but not neurofibrosarcoma-) derived SC, we have concluded that a combination of assays such as the BrdU and PCNA assays, rather than flow cytometry, as proposed, can best help us to correlate cell cycle stage with K current profile.

KEY RESEARCH ACCOMPLISHMENTS

K current types described:

Schwann cells (SC) cultured from neurofibrosarcomas had large, delayed rectifier (DR) K currents. These K currents, termed tumored K currents, were also found in SC cultured from neurofibromas, although other K current types were also present in neurofibroma-derived SC. Some normal, dividing SC had delayed rectifier (DR) K currents thought previously to be characteristic only of tumored SC. But the DR currents of normal SC were smaller in amplitude than in any tumored cell culture. Mean K current amplitudes of all normal SC cultures were significantly different than mean current amplitudes of all neurofibrosarcoma cell cultures. K current amplitudes in one normal culture were significantly different from those in the plexiform neurofibroma. K current amplitudes in the cutaneous neurofibroma were not significantly different from those in any normal SC culture.

Effect of channel block:

Blocking tumored K current with K channel antagonists blocks proliferation, whether this K current is present in a dividing normal SC or a SC derived from a tumor. Blocking K current also depolarizes SC resting potential.

Effect of blocking ras:

A farnesyl transferase inhibitor (FTI-III) blocks proliferation, and, our data indicate that FTIs do not have a strong effect on K channel block.

REPORTABLE OUTCOMES

1. Submitted application for U.S. Army Medical Research and Materiel Command National Neurofibromatosis Program grant, July 2001. Preliminary data includes the experimental results from this award.
2. Support for PhD student Diana Gonzalez was included in a campus-wide application to the Sloan Foundation to support minority graduate student education at our campus of the University of Miami, 8-01. The outcome of this application is pending. Ms. Gonzalez was chosen for inclusion due to her research success and the opportunities for mentoring afforded by this award.

CONCLUSIONS

1. The K current profiles of neurofibroma-derived SC are different than those of neurofibrosarcoma-derived SC.
2. There is variability in the type of K current profile observed from cell to cell. Thus neurofibroma-derived SC K currents have some features in common with dividing, normal SC.

3. We hypothesize that the ion channel phenotypes of normal, neurofibroma-derived and neurofibrosarcoma-derived SC represent a continuum from A type currents, characteristic of quiescent normal SC, to large tumored K type, characteristic of neurofibrosarcoma SC. Some aspects of the K current profile of all these SC types may be dependent on whether or not the SC is proliferating, while others may be dependent on the neurofibromin status of the cells. Assessment of K current profiles and proliferation status of individual cells in neurofibroma-derived SC cultures (the lucifer yellow technique used above) is a technique that may be useful for explanation of some of the variability in K current types observed. Specific blockers of possible component currents of tumored K current can also be used to address this hypothesis.

4. Blocking tumored K current with K channel antagonists blocks proliferation, implicating this current, or a component of it, in the proliferative capability of SC. Blocking tumored K current also depolarizes SC resting potential, but we believe this is secondary to K channel block and not the cause of the inhibition of proliferation.

5. A farnesyl transferase inhibitor blocks proliferation, which suggests that ras has a role in the proliferative capability of normal and NF1 SC in tissue culture.

PERSONNEL INVOLVED IN THIS RESEARCH

1. Lynne A. Fieber, PhD, Principal Investigator
2. Diana Gonzalez, graduate student

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