Development of proliferation assays to explore the role of calcium activated potassium channels in cell proliferation

Sean Foster

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DEVELOPMENT OF PROLIFERATION ASSAYS TO EXPLORE
THE ROLE OF CALCIUM ACTIVATED POTASSIUM CHANNELS
IN CELL PROLIFERATION

by

Sean Foster

A thesis submitted in partial fulfillment of the requirements
for the degree of

Departmental Honors in Biology

University of Richmond Biology Department

Spring 2006

This thesis has been accepted as part of the honors requirements in the Department of Biology

Approved by

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Honors Advisor

Date 5/1/02
Potassium channels are frequently over-expressed in cancerous cell lines compared to their non-cancerous counterparts. In many instances, specific groups of these channels have been implicated as key factors in regulating the cancer cells' proliferation. We have characterized two methods of detecting changes in proliferation: a colorimetric assay measuring the conversion of a reagent to a soluble dye by metabolically active cells, and a biophysics assay based upon the changes in impedance that occur as cells increasingly cover a gold electrode. We then applied both techniques to the identification of important potassium channels in the proliferation of porcine endometrial gland (PEG) cell proliferation. Preliminary findings have implicated calcium-activated potassium channels of the small conductance subfamily (SK) and voltage-gated potassium channels of the Kv1 class as potentially important factors in PEG cell proliferation.

Development of proliferation assays to explore the role of Calcium Activated Potassium Channels in Cancer Cell proliferation

by Sean Foster

Research Mentor: Linda Boland, Ph.D.
Department of Biology

A thesis presented on the research conducted between the Spring and Fall semesters of 2005 to develop reliable means of monitoring cellular proliferation and to investigate the potassium channels involved in this process.
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This research was partially funded by grants from the Howard Hughes Medical Institute and the University of Richmond Arts and Sciences Dean’s Office. The author thanks Dr. Scott O’Grady from the University of Minnesota for the donation of PEG cells, and Dr. Valerie Kish for the donation of U87MG cells. Additional thanks to Dr. Krista Stenger for the use of her plate reader. Finally, thanks to Kaelyn Krook, Nicole Buell and Matt Hitchcock for advice and assistance with data interpretation.
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INTRODUCTION

Cancer cells possess the ability to proliferate in an uncontrolled fashion, disrupted from their usual state of balanced cell-division and programmed cell death, or apoptosis. Potassium channels are transmembrane proteins that regulate cellular membrane potential in all cell types. Some K⁺ channels are activated by membrane voltage changes, whereas others are activated by voltage changes plus increases in intracellular calcium concentration. Recent evidence suggests that a variety of tumor cells exhibit altered expression of potassium (K⁺) ion channels, and that these channels may play a role in the cells' ability to proliferate (Jäger et al., 2004; Parihar et al., 2003; Weaver et al., 2004). The ubiquity of these channels and their suggested role in proliferation make them a potentially useful target for pharmacologic attack.

Although a large number of fundamental studies have identified potassium channels as playing a role in proliferation, little has been done to elucidate the mechanism of action. It has been proposed that cells require a hyper-polarization before entering the G1 phase of the cell cycle. In support of this theory, a 1972 study of Baby Hamster Kidney cells it was found that cell proliferation was inversely correlated to external potassium concentration (Orr et al., 1972). Externally applied potassium induces the influx of potassium ions as the cell nears its potassium equilibrium potential, in addition to decreasing the ability of potassium to efflux and stimulate hyperpolarization. Similar studies have been performed utilizing human astrocytomas and neuroblastomas (Lee et al., 1993) as well as early G1 stage Chinese Hamster V79 cells (Stambrook et al., 1973). Based on such studies, Wonderlin and Strobl developed a hyperpolarization-threshold model of K⁺ channel influence. According to this hypothesis, if K⁺ channels
must open before entrance into a particular phase of the cell cycle, then endogenous signaling pathways or externally applied drugs might inhibit passage through the control point by lowering the likelihood of the $K^+$ channels being open. Over-expression of these regulatory $K^+$ channels, however, could allow that only a small fraction of the expressed channels must open to reach the hyperpolarization threshold needed for passage. In such instances, the ability of either endogenous pathways or inhibitory drugs to regulate passage through the control point would be diminished even with no change in the activity of signal pathways (Wonderlin and Strobl, 1996). This theory provides just one explanation of how over-expression of a population of $K^+$ channels could result in enhanced proliferation.

Calcium is a second messenger involved in a vast array of cellular functions. As such, regulation of intracellular calcium concentration is a highly controlled process. To help maintain this delicate balance, many cells have evolved calcium-sensitive potassium channels. These channels enable a cell to maintain a hyperpolarized potential even after the influx of positively charged calcium ions. A growing body of work has indicated that members of the calcium-activated potassium channel super-family ($K_{Ca}$) may play a regulatory role in progression though the cell cycle. While little has been uncovered as to the mechanism of this action, it has been demonstrated numerous times that blockage of specific $K_{Ca}$ with channel toxins induces decreased proliferation rates. To date, this effect has been noted in cancerous lines from a variety of origins, including the pancreas, prostate, breast, brain, and muscle (Jäger et al., 2004; Parihar et al., 2003; Ouadid-Ahidouch et al., 2004).
The $K_{Ca}$ family has been relatively well characterized, largely due to the availability of highly selective channel blocking agents. The ion channel family is currently divided into three subfamilies based on the single channel conductance. Channels with the largest conductance are termed BK, and show the greatest voltage dependence. BK channels are selectively blocked by the toxin iberiotoxin, and non-selectively blocked by charybdotoxin, both of which are derived from scorpions. Channels with intermediate and smaller currents are termed IK and SK, respectively. Members of the IK family are highly specific targets of the synthetic, anti-fungal agent clotrimazole, and are non-selectively blocked by charybdotoxin. Members of the SK family are selectively blocked by a toxin in bee venom, apamin.

Studies investigating the role of $K_{Ca}$ channels in proliferation have indicated that the relative importance of the various subfamilies varies between cell lines. In human pancreatic cancer cells it was demonstrated that IK channel agonists induced a proliferation enhancement that was negated by addition of the IK channel blockers clotrimazole and charybdotoxin (Jager et al., 2004). Similarly, Parihar and colleagues (2003) found that both clotrimazole and tram-34, another IK channel blocker, inhibited proliferation of human prostate cancer cells in a dose dependent manner. In an investigation of human D54MG malignant glioma cells, serum starvation induced an up-regulation of BK channel surface expression, as measured by cell counting with a hemacytometer (Weaver et al., 2004). Iberiotoxin, a specific blocker of BK channels, induced cell death in serum-starved cells but was without effect on cell growth measured in normal serum-containing media. Because serum starvation may mimic the nutrient-depleted environment within a growing tumor, these results suggest that BK channels
may be required for the survival of cells in suboptimal conditions such as might be found at the center of a tumor in vivo (Weaver et al., 2004).

Understanding the factors that stimulate a cell to over-express K⁺ channels could offer the potential of more effective therapeutic targets than the channels themselves. Furthermore, such an understanding could provide key insights into the circumstances under which particular treatments might prove effective. For example, recent evidence has indicated that female steroid hormones may stimulate proliferation in a variety of cell lines. Of particular focus is the hormone 17-β-Estradiol, the primary human estrogen found in vivo. This hormone has been shown to alter cellular proliferation in a variety of cell lines. Interestingly, several groups have demonstrated that the effect of this hormone is at least partially accomplished through modulation of potassium channels of the Kₐ family. A 2002 study of neurons in the guinea pig brain indicated that 17β-estradiol may be responsible up-regulating the expression of SK3, a member of the SK family, thus indicating a genomic effect (Bosch et al., 2002). Conversely, a study of human breast cancer cells found that 17β-estradiol exerts its proliferation-enhancing effect by activating BK channels independently of the estrogen receptor, suggesting a non-genomic effect (Coiret et al., 2005). This finding might be explained by the results of an earlier study which demonstrated that estrogen activates BK channels in vascular smooth muscle by binding the beta subunit at an extra-cellular site (Valverde et al., 1999).

The porcine endometrial gland (PEG) cell line is a novel line that has been shown to possess channels in the Kₐ family (Deachapunya and Grady, unpublished data). The cell line is not cancerous and shows contact inhibition, but has been immortalized in
culture to proliferate continuously. Cells of this origin are particularly suited to the study of potassium channels, as it has been shown that potassium levels in pig uterine fluid are several fold higher than in the plasma, and that sodium levels are significantly lower than in plasma (cited in Deachapunya and Grady, 1998). These findings suggest that potassium channels may play a particularly important role in regulating the precise conditions necessary to create an optimal environment for embryo implantation and fetal growth.

In addition, the origin of PEG cells makes them a potentially useful system for studying the effects of hormone stimulation on ion channel function. This relationship has recently become the subject of much study in light of a wealth of research indicating a role for hormonal stimulation on cancer growth (Suzuki et al., 2005; Gaducci et al., 2005). In a recent review, the possible relationship between endometriosis and breast and ovarian cancers has been summarized. After investigating epidemiological data linking these conditions and exploring recent findings which indicate a role for inflammation and sex-steroid hormones in promoting these cancers, the authors suggest that endometriosis may be increasing the likelihood of the cancers by stimulating enhancement of hormone production and inflammatory response (Ness and Mudogno, 2006). Understanding the factors at work in regulating PEG proliferation as compared to factors regulating cancerous cell proliferation may help us to elucidate the specific factors at work in conveying cancerous cells their unique abilities. The MCF-7 cell line is derived from an adenocarcinoma in the breast. The line has been well characterized and has been featured in a significant number of publications over the last twenty years. It has been shown to express both IK and BK channels, and its proliferation has been inhibited by application
of the IK channel blocker clotrimazole (Ouadid-Ahidouch et al., 2004; Roger et al., 2004). Additionally, recent work has suggested that these channels show sensitivity to estrogen stimulation, as indicated by a reversible enhancement of current amplitude that occurs when the hormone is applied during patch-clamp studies (Coiret et al., 2005).

Due to the large variety of K⁺ channel families implicated in cancer cell proliferation, it is desirable to develop techniques capable of quickly and reliably screening a variety of potential channels for a role in proliferation. While there are many established cell counting procedures, the time-consuming nature of such protocols limits their usefulness in broadly identifying particular channels in uncharacterized cell lines. For this reason, a primary goal of the present research was the development of techniques capable of implicating particular ion channels in proliferation with both qualitative and quantitative data. Once this goal was accomplished, the project sought to conduct preliminary investigations into the role of potassium channels in PEG cell proliferation using the developed techniques.

2. Materials and methods

2.1 Cell Culture

The initial Porcine Endometrial Gland (PEG) cells were a gift from the lab of Dr. Scott O'Grady at the University of Minnesota. The PEG line is derived from uterine epithelium of the domestic pig and has been immortalized for continued cell division in culture. After proliferating to a confluent monolayer the cells demonstrate contact inhibition of proliferation as is typical of non-cancerous epithelial cells. PEG cells were cultured in Dubelco's Modified Eagle's Medium (DMEM, high glucose, pyridoxine HCl)
supplemented with 2 mM L-Glutamine, 0.1% insulin, 1% kanamycin sulfate, 1% penicillin-streptomycin, 1% MEM non-essential amino acids (NEAA) and 5% fetal bovine serum (FBS, Sigma).

The MCF-7 cell line was obtained from American Type Culture Collection (Rockville, Maryland, USA). The line is derived from a metastatic site in the pleural effusion, originating from an adenocarcinoma in the breast. MCF-7 cells were cultured in Eagle’s Minimum Essential Medium (EMEM with EBSS, NEAA and sodium pyruvate) supplemented with 1% penicillin-streptomycin, 1% L-glutamine, 0.2% insulin and 5% FBS.

The U87MG glioma cell line was a gift from the lab of Dr. Valerie Kish at the University of Richmond. Cells were cultured in Dubelco’s Modified Eagle’s Medium (DMEM, high glucose, pyridoxine HCl) supplemented with 2mM L-glutamine, 1% gentamycin sulfate, 1% penicillin-streptomycin, 1% NEAA and 5% FBS.

All cells were grown at 37°C in an atmosphere of saturating humidity and 5% carbon dioxide.

2.2 Colorimetric Assay

The WST-1 reagent (Roche) is a tetrazolium salt that is cleaved by mitochondrial dehydrogenases in metabolically active cells to form a soluble formazan dye according to the following reaction:

![Chemical reaction diagram](image)
All experiments with the WST1 reagent were performed in 96 well plates using a maximum volume of 200 µL per well. The reagent was added directly to media and, after gentle shaking for one minute, absorbance measurements were made with an ELISA plate reader between 0.5 and 4 hours after reagent addition. Per the manufacturer's instructions, absorbance was measured at 450nm with a 595 nm reference wavelength.

2.3 Biosensor Assay

The Electric Cell-Substrate Impedance Sensing (ECIS) array was obtained from Applied Biophysics (Troy, NY, USA). The array measures changes in the impedance to current flow between a reference electrode and small electrodes embedded in the floor of up to sixteen small wells. Individual wells were inoculated with a maximum volume of 400 µL of cell suspension. After inoculation of wells, cells were allowed a 24 hour period to adhere to the substratum prior to impedance measurement. Cells were inoculated in their usual growth media supplemented with experimental conditions as indicated. Unless otherwise indicated, media was exchanged every 24 hours.

2.4 Preparation of toxins

All toxin solutions were prepared from concentrated frozen stock solutions within 24 hours of the initial use for a given experiment. Unless otherwise noted, toxin solutions were prepared by diluting a concentrated stock with the appropriate growth media specific to the cell line being used. Experimental solutions were maintained at 4°C and were only opened until sterile conditions using proper aseptic technique. No toxin solutions were used for longer than one week after unfreezing.
Both charybdoxin and iberiotoxin were dissolved in sterile water and stored in aliquots at 20 µM. Per manufacturer's specifications apamin was dissolved in 10% acetic acid solution and stored in aliquots at 400 µM. Clotrimazole was dissolved in sterile water and stored in aliquots at 2 mM. Dendrotoxin-K was dissolved in sterile water and stored in aliquots at 30 µM.

3. Results and Discussion

3.1 Conditions for measuring proliferation with the WST1 reagent

To determine the optimal wavelength for monitoring formazan dye production, absorption spectra were obtained for the WST-1 reagent before and after metabolic processing, as well as for the growth media alone. Results indicate that only the formazan dye shows a significant peak between 400 and 500nm, with the un-metabolized reagent and media showing peaks in the 300 nm range (Figure 1). The lambda max for formazan appears to be approximately 440 nm, and no samples showed any absorbance at wavelengths greater than 500 nm. Although the lambda max of 440 nm does not exactly match the 450 nm filter available with our plate reader, the lack of any absorbance by media or un-metabolized reagent and the still-significant absorbance by the formazan product make 450 nm an acceptable wavelength with which to monitor dye production.
Characterization of the dynamic range of the WST-1 reagent with U87MG cells demonstrates an approximately linear range between 2500 and 50,000 cells in a 200 µL sample (Figure 2). The use of cell numbers below 5000 is likely unreliable, however, since dilutions to this density are difficult to count and are subject to error. When the incubation time before plate reading exceeds one hour the detection range appears to diminish, resulting in a smaller linear dynamic range and decreased ability to distinguish between the concentrations that were tested (Figures 2 and 3). This decrease in the linear dynamic range results from saturation of the absorbance signal, indicating that so much light is being absorbed that the differences in cell number can no longer be detected. Thus, it appears that optimal conditions for obtaining a broad detection range may be achieved by incubating cell cultures with WST-1 reagent for one hour, and by plating

![Figure 2. Determination of linear dynamic range for WST-1 proliferation assay. Figure depicts absorbance values at 450nm over a range of cell numbers at 30, 60, 120, and 240 minutes after the addition of WST-1 reagent. Data points represent the mean of three replicates.](image)

![Figure 3. Determination of optimal reading time after the addition of WST-1 reagent. Absorbance of various numbers of PEG cells taken at time points between 30 and 240 minutes. Data points represent the mean of three replicates.](image)
cells at a low density such that the maximum number of cells will not exceed 50,000 when the WST-1 dye is added.

Utilizing this information, the ability of the WST-1 assay to detect differences in proliferation was tested by comparing the absorbance signals from cells plated in either serum-free or 5% serum conditions. Because serum provides nutrients and hormones that are essential to the growth of most cells, it was hypothesized that serum starvation would induce a quiescence of growth, while the cells grown in 5% serum would proliferate normally. Thus, this experiment was selected to serve as a potentially simple means of assaying for differences in proliferation.

The wells were inoculated with 9200 cells in 200 µL of media with or without serum, and were allowed to grow over a four day period. The difference in growth that was anticipated between the two conditions is observed in readings taken on all three days following inoculation, with the cells plated in serum-containing media growing rapidly and those plated in serum-free conditions demonstrating negligible growth (Figure 4).

![Figure 4. Effect of serum starvation on U87MG proliferation. The figure depicts absorbance of U87MG cells plated at 9200 cells per well, with 0 or 5% serum concentration at 2, 3, and 4 days of growth under experimental conditions. Absorbance was measured 1 hour after WST-1 reagent addition. Data points represent mean of 3 replicates ± SE.](image-url)
3.2 Conditions for measuring proliferation with biosensor array

Although the ECIS biosensor array has not been well characterized for use in proliferation studies, our experiments have demonstrated that measuring impedance changes produces a normal growth curve when plated with PEG cells at low density (Figure 5). The figure demonstrates the lag phase, logarithmic growth phase and eventual plateau that are typical of immortalized cell proliferation. While it is possible to obtain growth curves when cells are plated over a range of densities, the rate at which a confluent monolayer is reached is proportional to initial plating density. Thus, in experiments seeking to differentiate between growth rates, it is desirable that the assay proceed over the course of several days such that the effects of experimental conditions are given the opportunity to demonstrate their effect in a detectable manner. Still, if the inoculation density is too low, the relatively small inoculation volume of 400 µL leaves room for random variation in cell density to significantly impact the number of cells inoculated in various wells. This effect could potentially mask the impact of an experimental treatment or indicate a growth change when, in fact, one has not occurred. For these reasons it was determined

![Figure 5. Standard growth curve for PEG cells proliferating under normal conditions in complete media. After inoculating at low density (approximately 25 cells/µL) the impedance to current flow was monitored for one week. Inset depicts conditions causing low impedance values (A) and high impedance values (B) depending upon the degree of electrode coverage.](image)
that the optimal inoculation density range for a 5 to 7 day growth assay is 25 to 50 cells per micro liter in a 400 µL aliquot (Figure 5).

In order to first demonstrate a detectable change in proliferation using the ECIS system, as well as to test the role of membrane potential as a factor in proliferation, the effect of membrane depolarization was explored by comparing the growth rate of cells in regular media and those grown in media containing 20 mM potassium chloride (Figure 6). As a control for osmotic effects, 40 mM sucrose was added to the media of cells grown in additional wells. Results of the experiment demonstrate that both the potassium chloride and sucrose conditions decrease the rate of proliferation of PEG cells to a degree that is detectable using the ECIS system. And though the sucrose-control demonstrates a significantly reduced proliferation, the potassium chloride treatment indicates an even greater impairment of proliferation. Although this does not specifically identify potassium channels as essential to proliferation, the results of this experiment demonstrate the regulation of membrane potential is critical to the normal proliferation of these cells. Because potassium channels are key regulators of membrane potential, this provides a justification for narrowing the field of study to particular
channel groups, such as the \( K_{Ca} \) family. Furthermore, these results indicate that the ECIS system is applicable to proliferation studies using the PEG cells.

To determine whether the use of the ECIS system was limited to epithelial-like cells that form confluent monolayers, the ability of the system to display a growth curve for MCF7 breast cancer cells was assessed (Figure 7). MCF7 cells were plated at approximately 80 cells per microliter and were allowed to grow for four days under normal growth conditions. As a control, PEG cells were plated at low density (~40 cells/µL) and simultaneously monitored. The results indicate no change in impedance in the wells containing MCF7 cells, while the wells containing PEG cells show a typical growth curve. To rule out the possibility that the MCF7 cells were absent from the wells or not proliferating, all wells were visually inspected after the experiment had concluded. Inspection did, in fact, demonstrate that MCF7 cells had proliferated normally. These results suggest that the ECIS biosensor assay is limited to epithelial like cells in the study of proliferation.

![Graph](image)

**Figure 7.** The inability of the biosensor array to monitor proliferation of MCF7 cells. MCF7 cells were plated at medium density (~80 cells/µL) and were allowed to grow for four days. The impedance changes in well inoculated with PEG cells at 50 cells/µL were monitored to serve as a positive control.
3.3 Potassium channels involved in PEG proliferation

In multiple experiments it was found that PEG cells grown in media containing 100 nM apamin showed impaired proliferation rates compared to cells grown in toxin-free media. This effect was demonstrated in ECIS experiments by an increased length of time required to reach a confluent monolayer, which is indicated by a plateau in impedance values (Figure 8). This effect was also demonstrated using the WST-1 assay, with PEG cells showing a decreased absorbance when incubated for three days in serum containing 100 nM apamin compared to control cells incubated in toxin-free media (Figure 9).

Although this effect is not observed at the 10 nM concentration, it is possible that slight decreases in proliferation are not measurable over the course of a three day proliferation assay.

Because apamin specifically targets channels in the SK subgroup, these results suggest that channels of this type may play a vital role in simulating PEG cell proliferation. The expression of SK channels in PEG cells has been demonstrated by O’Grady and Deachapunya (personal communication), further supporting this notion. Similar assays using a variety of IK and BK-specific channel blockers showed no
appreciable changes in proliferation, suggesting that only the SK subgroup of $K_{Ca}$
channels is involved in PEG proliferation (Data not shown).

In addition to applying $K_{Ca}$ channel blockers, the Kv1 specific channel blocker
dendrotoxin-K (DTX-K) was also applied to PEG cells to determine whether this sub-
family is implicated in proliferation. When plated at low density and
treated with 30 nM DTX-K, PEG
cells showed decreased rate of
growth in both ECIS and WST1
experiments. In the ECIS
experiment, this delay was indicated
by a failure to reach a confluent
monolayer through eight days of
growth, compared to control wells that reached confluency in an average of six days
(Figure 10). In an attempt to gain a dose response curve using the WST1 reagent,
treatments of 10, 30 and 100 nM DTX-
K were applied to PEG cells over the
course of three days. A dose response
curve is useful in demonstrating the
concentration dependence of an
observed effect and, in the case of an
ion channel toxin such as this,
supporting the notion that the effect is

Figure 9. Effect Apamin on PEG cell
proliferation (WST1). Cells were grown for 3
days in the absence or presence of apamin. Values
are the mean absorbance ($n = 4, \pm SE$) measured 3
days after seeding (1 hr after adding WST-1).

Figure 10. Effect of dendrotoxin-K on PEG
proliferation as indicated by ECIS system.
The plots represent the average impedance values
of wells treated with either 30 nM DTX-K or
regular media over a one week period ($n=2$).
dependent upon the degree of channel blockage. While the 10 nM concentration does not appear to impact the growth through three days, the results suggest that 30 and 100 nanomolar concentrations impair growth in a concentration dependent manner (Figure 11).

![Dose response curve for dendrotoxin-K inhibition of PEG cell growth. Values represent mean absorbance (n=4, ± SE) measured after one hour exposure to the WST-1 proliferation reagent. Absorbance was recorded after three days of treatment with experimental condition.](image)

**Figure 11.** Dose response curve for dendrotoxin-K inhibition of PEG cell growth. Values represent mean absorbance (n=4, ± SE) measured after one hour exposure to the WST-1 proliferation reagent. Absorbance was recorded after three days of treatment with experimental condition.

4. Conclusions

The present work has demonstrated the general protocol and ideal conditions for monitoring cellular proliferation. The WST-1 colorimetric method has been shown to possess a workable dynamic range when cells are plated at low density. Using the observed linear dynamic range between 5000 and 50,000 cells, it is estimated that a well inoculated at a density of approximately 50 cell/µL, or 10,000 cells in the standard 200 µL volume, could endure between 2 to 3 doubling events before saturation of the absorbance signal would be observed. It is therefore important to consider the doubling time of the cell line being used when determining the length of incubation before WST-1
reagent is added and absorbance is recorded. For example, it has been determined that MCF7 cells possess a doubling time of approximately 48 hours (Krook and Buell, 2006). With this in mind, a useful WST-1 assay using these cells would allow the treatments to incubate for a minimum of four days and a maximum of six days if inoculated at 50 cells/µL. A cell line with a more rapid doubling time, such as the PEG cells, would have to be read several days earlier to achieve the same confidence that readings were within the linear dynamic range of the technique. The number of allowable doublings could be increased by a lower inoculation density; however, it becomes increasingly difficult to ensure homogeneity in a solution significantly more dilute than this, thereby increasing the opportunity for error. The ability of the WST-1 reagent to function with a variety of cell types is a useful characteristic of the technique.

The ECIS array has been shown to effectively detect cellular proliferation by monitoring changes in impedance values as cells grow and cover the gold electrode surfaces within the array wells. While the ECIS technology has not been widely used for proliferation studies, other studies have supported its use for this purpose (Lundien et al., 2002). Unlike the WST-1 reagent, this method of proliferation detection is limited to cell lines that form confluent monolayers. Thus, this technology is useful for studies with cell lines such as the PEG line, but is not useful for studying lines like the U87MG and MCF7 lines. It has been previously shown that the changes in impedance associated with cell proliferation in the ECIS system result from the formation of tight junctions that restrict current flow between cells (Wegener et al., 2000). As such, the lack of ability to detect proliferation in tumor-forming lines like MCF7 and U87MG may be attributed to the scarcity of tight junctions between cells, thus allowing the electrolytes to flow between
electrode and counter-electrode without significant changes in impedance even when electrodes are partially covered.

In addition to providing basic information about the utility of the proliferation assays, some initial data has been gathered implicating two potassium channel groups as potentially key regulators of cell proliferation. The inhibition of small conductance, calcium-activated potassium channel group (SK) with the bee-venom toxin apamin has been shown to cause decreased proliferation in assays utilizing both proliferation monitoring techniques. Similarly, application of DTX-K, a snake venom that specifically inhibits voltage gated channels of the Kv1 family, has been shown to impair proliferation in both WST-1 and ECIS assays.

Although this work has provided initial data implicating channels in proliferation, it is necessary that the assays be replicated in order to confidently assert a significant role. Once this has been done, research should aim at uncovering the mechanism or pathway by which the potassium channel exerts its influence. The use of flow cytometry to determine the distribution of cells between the various stages of the cell cycle has become a popular way of gaining insight into the mechanism of action of proliferation halting reagents and toxins. Using this technique it has been demonstrated that treating MCF7 cells arrested late in the G1 or S phases with clotrimazole, which was shown to inhibit proliferation of these cells, induced significant membrane depolarization, but that this effect was not observed when cells arrested in early G1 were treated. From this, the authors asserted a key role for IK channels, which are specifically blocked by clotrimazole, in regulating the membrane potential of MCF7 cells during the G1/S transition (Ouadid-Ahidouch et al., 2004). Conducting such a synchronization study with
PEG cells treated with apamin could help to elucidate the precise role that SK channels play in PEG proliferation.

The role of upstream messengers such as steroid hormones and growth factors should also be explored, as an abundance of recent data has implicated a relationship between such factors and potassium channels (Suzuki et al., 2005; Gaducci et al., 2005). Possible mechanisms of exploring this role could include the expression of channels in oocytes and measurement of current changes associated with hormonal stimulation, a powerful electrophysiological technique that has already been used to examine the effect of estrogen on BK channels isolated from MCF7 cells (Coiret et al., 2005).
Literature Cited


