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Naringenin is a novel inhibitor of *Dictyostelium* cell proliferation and cell migration

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Abstract

Naringenin is a flavanone compound that alters critical cellular processes such as cell multiplication, glucose uptake, and mitochondrial activity. In this study, we used the social amoeba, *Dictyostelium discoideum*, as a model system for examining the cellular processes and signaling pathways affected by naringenin. We found that naringenin inhibited *Dictyostelium* cell division in a dose-dependent manner ($IC_{50} \sim 20$ μM). Assays of *Dictyostelium* chemotaxis and multicellular development revealed that naringenin possesses a previously unrecognized ability to suppress amoeboid cell motility. We also found that naringenin, which is known to inhibit phosphatidylinositol 3-kinase activity, had no apparent effect on phosphatidylinositol 3,4,5-trisphosphate synthesis in live *Dictyostelium* cells; suggesting that this compound suppresses cell growth and migration via alternative signaling pathways. In another context, the discoveries described here highlight the value of using the *Dictyostelium* model system for identifying and characterizing the mechanisms by which naringenin, and related compounds, exert their effects on eukaryotic cells.

Keywords: Cell migration; Cell proliferation; Phosphatidylinositol-3 kinase; Naringenin; *Dictyostelium*; Chemotaxis; Cell division; Flavonoids

The development and progression of cancer is a highly complex process involving unregulated cell growth (tumor formation) and cell migration (metastasis). Naringenin, a flavanone compound highly enriched in grapefruits, has been identified as a possible inhibitor of cell proliferation [1,2]; and thus has the potential to act as an anti-tumorigenic agent. However, the mechanism by which naringenin is able to suppress cell growth is still unclear since this compound appears to have multiple cellular targets including cytochrome P450 enzymes [3], the phosphatidylinositol-3 kinase (PI3K) pathway [4,5], glucose uptake pathways [5–7], among others [3]. In the studies described in this report, we employed the social amoeba, *Dictyostelium discoideum*, as a model system for examining the cellular processes and signaling pathways altered by exposure to naringenin.

Dictyostelium exists for much of its life cycle in a single-celled amoeboid state in which it feeds via phagocytic uptake of bacterial cells and multiplies mitotically. When the supply of nutrients has been exhausted, *Dictyostelium* cells become elongated and migrate via chemotaxis into aggregates; these aggregates then develop into multicellular fruiting bodies comprised of specialized cells performing distinct functions [8,9]. *Dictyostelium* has served as a powerful model system for identifying and characterizing the basic mechanisms driving cell division/multiplication [10–12], cell migration [13], and multicellular development [14,15]; many of these processes play critical roles in health and disease, and are either absent or less accessible for study in other model systems. Another advantage of the *Dictyostelium* system is that researchers are able to alter the expression of specific genes with relative ease, and then observe the effects of those genetic manipulations on the behavior of the cells [14–19]. Such studies in *Dictyostelium* have been instrumental in identifying and characterizing the pathway components regulating cell division, thus

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providing a foundation for understanding how such processes go away during the uncontrolled cell multiplication seen in tumor growth [11,18,20,21].

In this report, we show that naringenin suppresses *Dictyostelium* cell multiplication in a concentration-dependent manner, but is not cytotoxic to cells and does not induce alterations in cytokinesis. Our studies also show for the first time that naringenin is a potent inhibitor of eukaryotic cell motility, and thus has the potential to curb the uncontrolled cell migration exhibited by cancer cells. While previous studies demonstrate that naringenin can inhibit phosphatidylinositol 3-kinase activity in vitro [5,22], we report here that in live cells undergoing directed cell motility (chemotaxis), naringenin has no apparent effect on the synthesis of phosphatidylinositol 3,4,5-trisphosphate in response to chemoattractant stimulation of cells. Taken together, these results highlight the possibility that dietary intake of naringenin could protect against aberrant cell proliferation and/or migration related to the development of cancer. In another context, the discoveries described here underscore the value of using the *Dictyostelium* system as a means of identifying new cellular targets of naringenin and related compounds.

Materials and methods

Analysis of Dictyostelium cell growth. *Dictyostelium* cells (strain AX2) [23] were grown to confluence in 15 cm Petri dishes containing HL5 culture medium [24]. These cells were harvested and used to start 5×10^4 cells/ml cultures supplemented with naringenin (or other compounds, as indicated) and then grown with shaking at 185 rpm at 21 °C. A parallel culture lacking naringenin was grown as a positive control for normal *Dictyostelium* cell growth in suspension culture. All cultures, including the positive control culture, contained 0.7% DMSO, which was used as the solvent for naringenin stock solutions. At 24 h intervals (up to 96 h), an aliquot of each culture was removed and the cells were counted on a hemacytometer to determine cell density; these values were plotted for comparison of growth rates/levels among the different culture conditions.

Determination of cytokinesis defects. To analyze the number of nuclei per *Dictyostelium* cell after 96 h of growth in suspension ±100 μM naringenin, 1×10^4 cells were spotted on a glass coverslip and allowed to adhere for 20 min. The culture medium was then replaced with buffer (20 mM MES, pH 6.8, 0.2 mM $CaCl_2$, and 2 mM $MgCl_2$) and cells were incubated for 30 min at 21 °C. The cells were incubated for another 30 min with fresh buffer, followed by another 15 min incubation in buffer containing 20 μg/ml DAPI (4′,6-diamidino-2-phenylindole) to stain cell nuclei. The cells were then washed twice with buffer (no DAPI) and then visualized via epifluorescence using an Olympus IX81 motorized microscope system with an UPlanFL 20× objective lens (NA 1.3) to visualize the number of nuclei per cells. Myosin II-null cells (strain HS1 [25]) were included as a positive control for the cytokinesis defect.

Viability assays. Cells were analyzed for viability after 72 h of growth in suspension cultures containing 200 μM naringenin or an equal volume of vehicle (DMSO). Positive control cultures were grown in the same manner but with no additions. Cell viability was determined using the method of Alexander et al. [26]. Briefly, a 1:1000 dilution of each *Dictyostelium* culture was mixed with an aliquot of *Klebsiella aerogenes* bacteria and then spread with sterile glass beads over SM5 agar in 20 mm wells of a 12-well flat-bottomed plate (Becton–Dickinson Labware). The plate was incubated at room temperature until clear areas (“plaques”) became visible (usually 3–5 days). Each plaque represents the growth of one viable cell. The plates were scanned using a Hewlett Packard 7400 C flatbed scanner and the number of plaques was quantified manually from images processed using Image Pro-Plus™ software (Media Cybernetics, Inc.).

Analysis of multicellular development. *Dictyostelium* cells were first grown to confluence on 15 cm plates in HL5 medium lacking naringenin. Approximately 1×10^8 cells in log phase growth were harvested and washed three times with starvation buffer (20 mM MES, pH 6.8, 0.2 mM $CaCl_2$, and 2 mM $MgCl_2$), and the final pellet was re-suspended in 1 ml of starvation buffer containing either 50 μM naringenin or an equal volume of DMSO (vehicle) and incubated for 60 min at 21 °C with gentle shaking (150 rpm). The cell slurry was then spread evenly on a Whatman #50 filter pad soaked with starvation buffer sitting on top of two Whatman #3 filters saturated with starvation buffer. The cells were allowed to undergo multicellular development and the progress of development (i.e., fruiting body formation) was assessed at 48–96 h. Images were collected using a Nikon DXM1200 High Definition Cooled Color Digital Camera mounted on a Nikon SMZ1000 Zoom Stereomicroscope.

Folic acid chemotaxis assays. The effect of naringenin on *Dictyostelium* chemotaxis was analyzed using the under-agarose cell migration assay as described by Laevsky and Knecht [27], with no significant modifications. Briefly, *Dictyostelium* AX2 cells were grown to log phase on 10 cm Petri plates in HL5 medium (no naringenin). The culture medium was then aspirated off, and the attached cells were collected in fresh HL5 medium (about 1 ml) to a density of 1×10^7 cells/ml and then incubated (60 min at 21 °C with gentle shaking—150 rpm) in the presence of either 80 μM naringenin (in DMSO) or the same volume of DMSO alone (untreated). After incubation, approximately 1×10^6 cells of the naringenin treated and untreated cells were deposited in opposite troughs of the same under-agarose assay plate. Under-agarose assay plates (0.5% agarose; Fisher Bioscience) were prepared exactly as described by Laevsky and Knecht [27] with a central trough containing the chemoattractant folic acid (100 μM; Sigma–Aldrich) and two peripheral troughs spaced equidistant from the central trough and opposite of each other. Folic acid was applied to the central trough and was allowed to diffuse through the agarose for approximately 1 h prior to adding the cells to the peripheral wells. The cells were allowed to chemotax under the agarose towards the source of folic acid for 4 h and then examined using an Olympus IX70 inverted microscope (10× objective lens). Phase-contrast images were collected with a Photometrics CoolSNAP™ camera and then processed using Image Pro-Plus™ software (Media Cybernetics, Inc.). Chemotaxis was quantified by counting the number of cells that have migrated into a 450 μm × 350 μm area along troughs containing either treated or untreated cells. Cells within this area are migrating under the agarose towards the source of folic acid.

GFP-CRAC translocation assays. *Dictyostelium* AX2 cells harboring the pWf1-CRAC-GFP plasmid for the expression of GFP-tagged CRAC fusion protein (cytosolic regulator of adenylyl cyclase; generously provided by Dr. Carole Parent, NIH [28]) were grown on 15 cm plastic Petri dishes containing liquid HL5 medium supplemented with 10 μg/ml Geneticin (G418; Gibco). The GFP-tagged CRAC protein (GFP-CRAC) binds specifically to phosphatidylinositol 3,4,5-trisphosphate (PIP_3) and has been used extensively as an in vivo sensor for PI3K-mediated synthesis of PIP_3 during *Dictyostelium* chemotaxis [29]. For our experiments, cells were prepared for chemoattractant response studies by inducing development to the aggregation stage, as has been described previously [28,30]. Briefly, growth phase *Dictyostelium* cells were collected by centrifugation, washed twice in starvation buffer, and then re-suspended to a density of 4×10^7 cells/ml. The cell suspension was shaken (150 rpm) for 2 h at 21 °C and then pulsed every 6 min with 100 nM cAMP for 6–8 h using a Branson syringe pump to apply the concentrated cAMP drip. Two hours prior to preparing the cells for cAMP stimulation, 200 μM naringenin (final concentration) or an equivalent volume of vehicle (DMSO) was added to the cell slurry. After 2 h of incubation, the cells were then brought to a ‘primed’ stimulation state by adding caffeine to a final concentration of 2.5 mM and then shaken for an additional 30 min. An aliquot of 5×10^4 cells was then spotted on a glass coverslip and the cells were allowed to adhere for 20 min. Subsequently, the cells were stimulated with 10 μM cAMP, and images were collected before the addition of cAMP (0 s) and 15 s after application of the chemoattractant. The cells were visualized via epifluorescence using an Olympus IX81 motorized

microscope system with an UPlanFL 40× objective lens (NA 1.3), and images were processed using Image Pro-Plus™ software (Media Cybernetics, Inc.).

Results and discussion

Naringenin inhibits Dictyostelium cell proliferation

Naringenin has been shown to inhibit cellular proliferation in a variety of cell types [2], and thus may have the potential to act as an anti-tumorigenic agent in vivo. To explore the anti-proliferative effects of naringenin further, we analyzed the growth of the amoeboid eukaryotic model organism, *D. discoideum*, in the presence and absence of different concentrations of naringenin. Our results revealed that naringenin indeed suppresses *Dictyostelium* cell growth in a concentration-dependent manner with an IC_{50} of ~20 μM (Fig. 1A). It is noteworthy that the concentrations of naringenin that inhibit *Dictyostelium* growth are similar to the plasma levels (0.7–14 μM) of naringenin detected in individuals who have consumed approximately 200 mg naringenin in grapefruit juice [31]. Interestingly, *Dictyostelium* cells are still able to multiply for a short period of time after the addition of naringenin to the culture medium, perhaps indicating that a critical intracellular concentration of naringenin must be reached before cell growth is suppressed. Complementary experiments revealed that naringenin inhibits *Dictyostelium* cell growth more potently than compounds from other classes of flavonoids (Fig. 1B), suggesting that naringenin may be more effective at inhibiting uncontrolled cell proliferation related to tumor formation. Collectively, our observations of the growth-inhibiting effects of naringenin on *Dictyostelium* are consistent with those reported for other eukaryotic cell types [2], and suggest that the targets of naringenin activity are conserved among a broad variety of eukaryotic cell types. By extension, our results also demonstrate that *Dictyostelium* is a useful model system for studying the effects of naringenin on fundamental cellular processes.

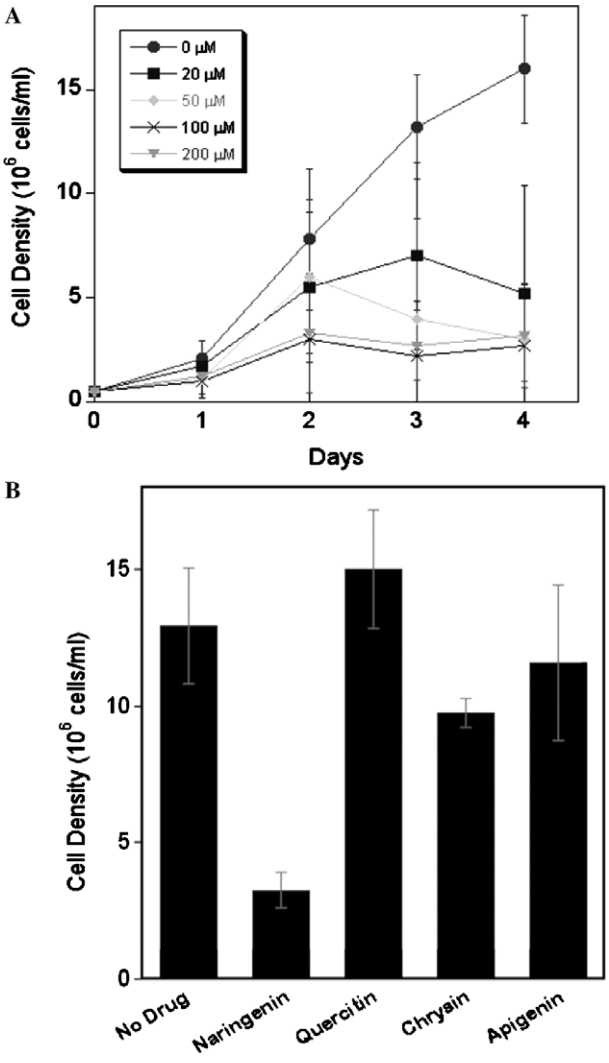

Fig. 1. Naringenin inhibits *Dictyostelium* cell proliferation. (A) Growth rates were determined for *Dictyostelium* cells (AX2 strain) cultured in the presence of different concentrations of naringenin—20 μM (■), 50 μM (◆), 100 μM (×), 200 μM (▼), or no naringenin (●) as described in the Materials and methods section. (B) *Dictyostelium* cell density was assessed after 3 days in shaking culture in the presence of each flavonoid compound (200 μM) as indicated below each bar in the graph. Each plotted point or bar represents the average cell density for that given condition from at least four separate experiments. The error bars represent the standard error of each mean.

Naringenin-treated Dictyostelium cells are not defective in cytokinesis

While naringenin has been shown to suppress the growth of cells from a variety of cancer cell lines, it is still unclear if the anti-proliferative effects of this compound arise from disruption of the cell cycle or via toxic effects on the cells [32,33]. Thus at this time, the possibility exists that the anti-proliferative effects of naringenin may be due to alterations in the ability of the cells to undergo normal cytokinesis, the last phase of the cell cycle. *Dictyostelium* represents an excellent system to explore this hypothesis since cytokinesis defects in *Dictyostelium* can be easily identified as a dramatic increase in the number of large, multinucleated cells that form when *Dictyostelium* is grown in suspension culture. These characteristics of cytokinesis defects are readily apparent in positive control cells lacking myosin II (Fig. 2) [34]. While we have shown that a concentration of 100 μM naringenin greatly reduces *Dictyostelium* cell proliferation (Fig. 1A), we do not detect a corresponding increase in the number of large, multinucleated cells in suspension cultures containing that same concentration of naringenin (Fig. 2). Thus, it appears that the ability of naringenin to inhibit cell growth does not occur via alteration of normal cytokinesis, but instead is likely to occur prior to this event in the cell cycle. Studies are in progress to determine the specific phase of the *Dictyostelium* cell cycle that is affected by naringenin.

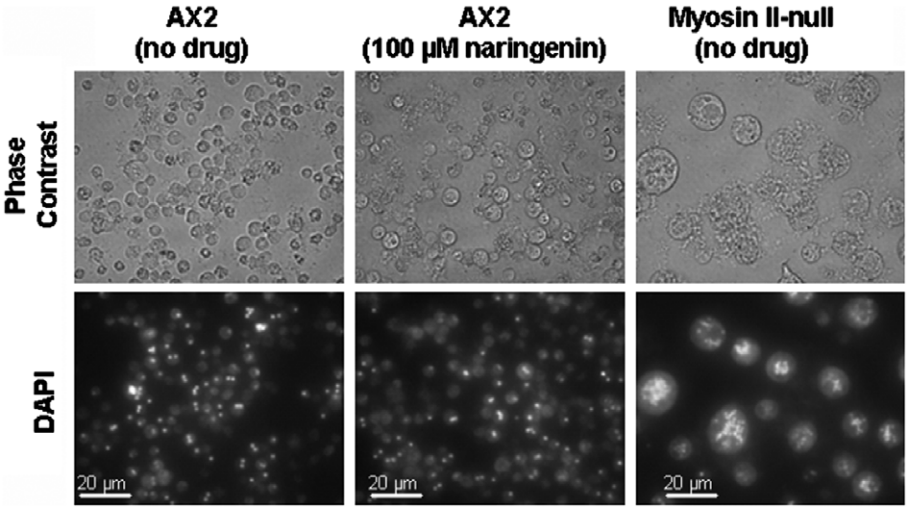

Fig. 2. Analysis of cytokinesis defects in *Dictyostelium* cells treated with naringenin. *Dictyostelium* cells were cultured in suspension for 4 days in the presence or absence of 100 μM naringenin as described in the Materials and methods section. Aliquots of the same number of cells were then collected and stained with DAPI to determine the number of nuclei/cell. The micrographs on the top row are phase contrast images of wildtype (AX2) cells that have been grown in the presence or absence of naringenin, and untreated myosin II-null cells that are defective in cytokinesis. The number of nuclei present in these cells was visualized via epifluorescent imaging of the DAPI-stained cells (bottom row).

Exposure to naringenin does not affect Dictyostelium cell viability

Since the suppression of *Dictyostelium* cell growth in the presence of naringenin could be simply due to drug toxicity, we next tested the viability of cells after they have been cultured for several days in the presence of a high concentration of naringenin (200 μM). To this end, we used the method of Alexander et al. [26] in which the same number of cells from different treatment populations (i.e., no drug, vehicle/DMSO, or 200 μM naringenin) was grown on *Klebsiella* bacterial lawns and the appearance of a cleared spot (plaque) after 3–5 days represents growth from a single viable cell. Our results revealed that the number of plaques formed by naringenin treated and untreated cells did not differ, indicating that cell viability is not compromised by exposure to naringenin (Fig. 3). It is particularly noteworthy that the effects of naringenin on *Dictyostelium* cell proliferation are not permanent, and thus are not cytotoxic, since transferring the cells to an environment devoid of naringenin (i.e., *Klebsiella* lawns) restores the ability of the cells to multiply. These results, along with our other findings showing that naringenin does not affect cytokinesis (Fig. 2), support the idea that naringenin suppresses cell proliferation by inhibiting the cell cycle at an early stage.

Naringenin inhibits Dictyostelium multicellular development

A hallmark of the cancer cell phenotype is uncontrolled cell migration (metastasis). While a number of studies support the notion that naringenin inhibits cell proliferation in a variety of cell types, the effect of naringenin on cell motility has not been explored in depth [35]. The *Dictyostelium* system provides an excellent opportunity to investigate the effect of naringenin on eukaryotic cell motility since *Dictyostelium* cells can be induced to undergo chemotactic migration in a consistent and highly reproducible manner in vitro. When a population of *Dictyostelium* cells is placed in an environment lacking nutrients, a small fraction of the cells will begin secreting cyclic adenosine monophosphate (cAMP). *Dictyostelium* cells respond to a gradient of cAMP by becoming highly polarized and then migrating into cellular streams that move towards the source of the chemoattractant. The chemotaxing cells coalesce to form a mound and then undergo further differentiation and development to form a multicellular fruiting body. *Dictyostelium* cells lacking the ability to undergo ''normal'' cell migration are often unable to develop beyond the initial aggregation and/or mound stages of development [9].

For our studies, *Dictyostelium* cells were first grown to log phase in the absence of naringenin, and then transferred to buffer containing either 50 μM naringenin or an equal volume of DMSO (vehicle). These cells were then spotted on filter pads for multicellular development. Results from this experiment revealed that cells allowed to undergo development in the absence of naringenin formed fruiting bodies within 3 to 4 days after they had been spotted on filters (Fig. 4; left column). In contrast, cells treated with naringenin were unable to develop beyond the mound stage of development (Fig. 4; right column), even after 7 days in starvation conditions. The inability of *Dictyostelium* cells to develop beyond the mound stage is characteristic of cells that are unable to undergo the highly regulated changes in the cytoskeleton that are necessary for proper cell migration [36], and suggests that naringenin may target similar processes in the cell.

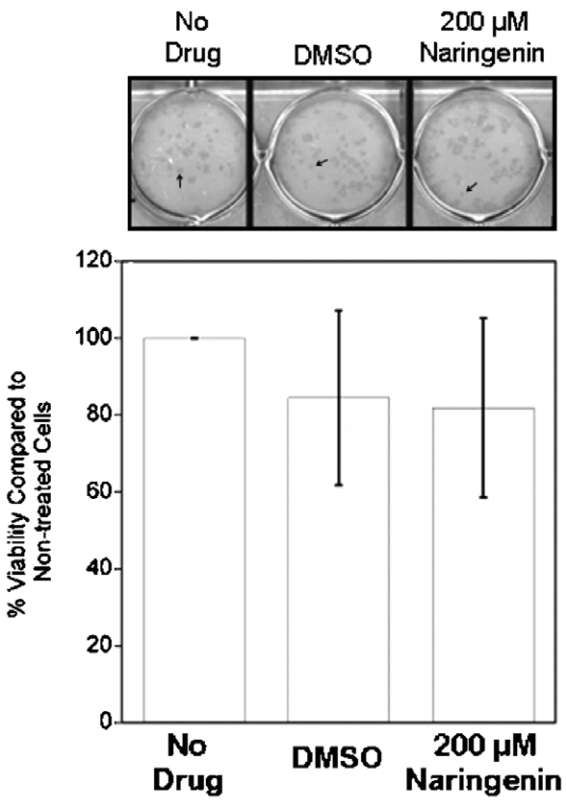

Fig. 3. Determination of the effects of naringenin on *Dictyostelium* cell viability. The viability of *Dictyostelium* cells cultured for 4 days in the presence or absence of 200 μM naringenin was determined by plating the same number of cells on a lawn of *Klebsiella* bacteria (lacking naringenin) and incubating at 21 °C for 2 to 3 days, as described in the Materials and methods section. A representative row of plate wells showing the formation of plaques by viable *Dictyostelium* cells is provided above the graph. The growth conditions (±naringenin or DMSO) prior to plating are provided above each well; arrows are provided to indicate a representative plaque from each well. The number of plaques formed was quantified and plotted (see bar graph) for each growth condition (indicated below the corresponding bar). Each bar represents the mean number of plaques formed from three separate experiments and the error bars are the standard errors of those means.

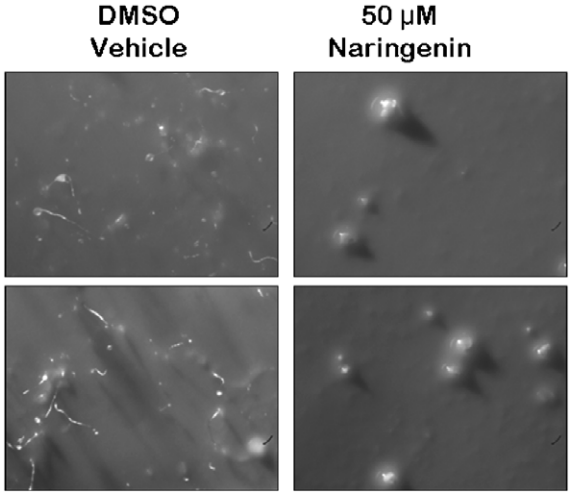

Fig. 4. *Dictyostelium* multicellular development in the presence of naringenin. Cells were cultured in HL5 medium to log phase of growth and then transferred to starvation buffer either containing or lacking 50 μM naringenin. After a 60 min incubation (21 °C), the *Dictyostelium* cells were prepared for multicellular development as described in Materials and methods. The images on the left show the formation of fruiting bodies by untreated cells; the top and bottom images are from separate experiments. In contrast, *Dictyostelium* cells incubated with 50 μM naringenin (images to the right) developed no further than the mound stage and did not form fruiting bodies.

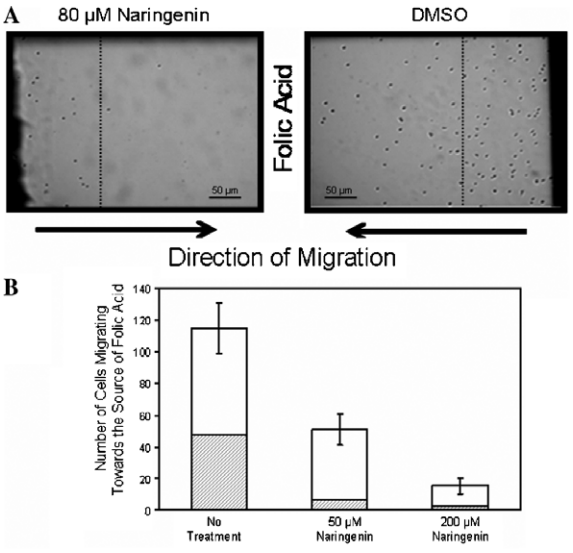

Fig. 5. The effects of naringenin on *Dictyostelium* cell migration. (A) Images of chemotaxing cells (±80 μM naringenin, as indicated above each image) were taken 4 h after adding the cells to the outer troughs of the under-agarose assay plate (see Materials and methods). The arrows indicate the direction of cell migration towards the source of folic acid, and the vertical dotted line in each image is placed approximately 150 μm from the point at which the cells enter the agarose. (B) Cell migration was quantified as described in Materials and methods by counting the number of cells migrating into the 450 μm × 350 μm area shown in (A) of this figure. The cell counts from four separate trials were averaged for each experimental condition and the resulting values were plotted in accompanying bar graph. The striped portion of each bar represents the number of cells migrating at least 150 μm towards the source of folic acid. The error bars represent the standard error of each mean.

Dictyostelium chemotaxis is inhibited by naringenin

To further explore the possibility that naringenin induces a cell motility defect in *Dictyostelium* cells, we performed under-agarose chemotaxis assays as described by Laevsky and Knecht [27]. With this method, vegetative *Dictyostelium* cells will migrate under a layer of agarose towards the chemoattractant folic acid. Using this assay, we found that cells exhibited greatly reduced chemotaxis towards folic acid when treated with 80 μM naringenin. In contrast, untreated cells exhibited robust migration towards the source of folic acid (Fig. 5A; compare left and right panels). Quantification of chemotaxis revealed that the inhibition of *Dictyostelium* cell motility is dependent on the concentration of naringenin, with nearly complete inhibition observed at 200 μM naringenin (Fig. 5B). Our results are particularly interesting since they represent the first

demonstration that naringenin has the ability to suppress eukaryotic cell migration. These findings contrast with those from Fenton and Hord [35] which indicate that flavonoids actually promote cell migration via the activation of matrix metalloproteinase activity and the resulting degradation of the extracellular matrix that can limit cell motility. *Dictyostelium* cell migration does not involve interaction with an extracellular matrix; thus our observations suggest that naringenin may disrupt the basic intracellular changes (i.e., F-actin reorganization, myosin II activity, etc.) required for directed cell motility [37].

Chemoattractant-induced stimulation of P3,4,5P$_3$ is not affected in Dictyostelium cells treated with naringenin

Previous studies have demonstrated that naringenin (and other flavonoids) can inhibit phosphatidylinositol 3-kinase (PI3K) activity [5,22]. Members of the PI3K family catalyze the synthesis of phosphatidylinositol 3,4,5-trisphosphate (PIP_3), an important second messenger that alters the activities of many downstream signaling molecules that function broadly in the regulation of cell growth and survival [38]. In *Dictyostelium*, PI3K plays a central role in defining the signaling events at the leading edge of cells migrating towards a source of chemoattractant [17]. The in vivo synthesis of PIP_3 in *Dictyostelium* can be monitored directly by expressing a GFP-tagged version of the protein CRAC (cytosolic regulator of adenylyl cyclase) which possesses a PH domain that binds specifically to PIP_3 in the cell [28]. When chemotaxis-competent *Dictyostelium* cells are exposed to a uniform, saturating concentration of cAMP, GFP-CRAC is rapidly recruited to the cell cortex, indicating the synthesis of PIP_3 at those sites. Studies reported elsewhere have demonstrated that chemoattractant-induced accumulation of GFP-CRAC to the cell cortex is inhibited in *Dictyostelium* cells treated with the PI3K inhibitor LY294002 [39].

To test the effects of naringenin on PI3K activity in vivo, we assayed live *Dictyostelium* cells for chemoattractant-stimulated synthesis of PIP_3 by monitoring the translocation of GFP-CRAC to the cell cortex in the presence and absence of naringenin. It is noteworthy that this study is the first to examine the effects of naringenin (or any other flavonoid compound) on the in vivo PI3K activity in any eukaryotic system. Our results revealed that treatment of cells with naringenin (up to 200 μM) did not alter the rapid increase in cortical PIP_3 synthesis that occurs upon exposure of *Dictyostelium* cells to cAMP (Fig. 6). We show here that *Dictyostelium* PI3K activity is not inhibited even at naringenin concentrations that suppress both cell division and chemotaxis. Our data also show that the early steps in the chemotaxis signaling pathways are still intact in *Dictyostelium* cells treated with naringenin; suggesting that naringenin targets unidentified distal events which are necessary for directed cell migration. Future studies will focus on using naringenin as a tool to further delineate the signaling components regulating cell migration.

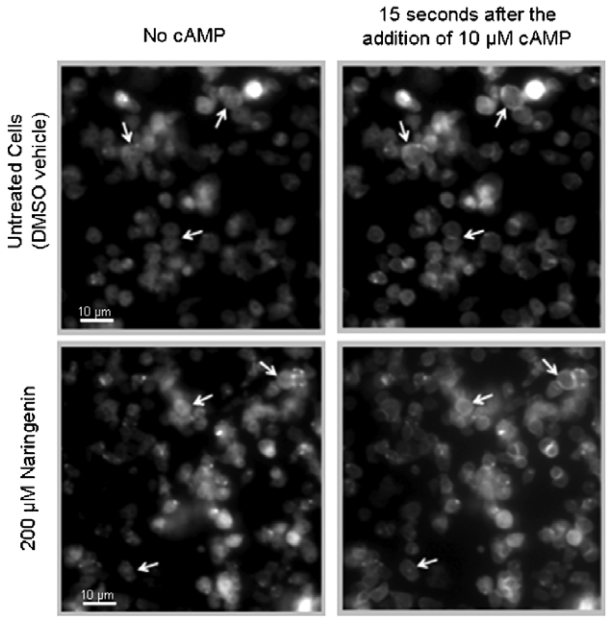

Fig. 6. Analysis of in vivo PI3K activity in live *Dictyostelium* cells treated with naringenin. Epifluorescence images were collected immediately before and 15 s after the addition of 10 μM cAMP to the cells. Chemoattractant (cAMP)-induced synthesis of PIP_3 by PI3K is reflected in the enrichment of GFP-CRAC fusion protein at the cell cortex (see arrows). These micrographs are representative of four independent experiments.

In summary, we have used the eukaryotic model organism *D. discoideum* to show for the first time that naringenin suppresses eukaryotic cell migration, as well as cell proliferation. These studies highlight the potential for using naringenin as a pharmacological agent to suppress both the tumorigenic and metastatic activities of cancer cells. The utility of the *Dictyostelium* system also provided the unique opportunity to assay the effect of naringenin on the in vivo activity of PI3K. These studies revealed that naringenin did not alter PI3K activity, but did impair processes such as chemotaxis and cell division that require PI3K activity, suggesting that naringenin may impose its effects on the cell by altering signaling components downstream of PI3K or via the inhibition of PI3K-independent pathways. Future studies will focus on identifying novel cellular activities and signal transduction pathway components targeted by naringenin and how these targets may be involved in the basic cellular processes that go awry in the cells exhibiting the cancer phenotype.

Acknowledgments

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