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Differential control of growth, cell cycle progression, and expression of NF-κB in human breast cancer cells MCF-7, MCF-10A, and MDA-MB-231 by ponicidin and oridonin, diterpenoids from the chinese herb *Rabdosia rubescens*

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Abstract

Ponicidin and oridonin are novel diterpenoids isolated from *Rabdosia rubescens*. We tested their effects in MCF-7 and MDA-MB-231 cells, as representing low and high invasive breast carcinoma, with normal MCF-10A cells. Clonogenicity and proliferation in MCF-7 cells were inhibited more significantly by ponicidin than oridonin, while the reverse was observed in MCF-10A cells. Ponicidin and oridonin induced S/G_2M arrest and G_1/S block in MCF-7 cells. In MCF-10A cells treated with either diterpenoid, induction of apoptosis was observed. Moreover, oridonin almost completely blocked MCF-10A progression from S to G_2/M phase; in contrast, ponicidin-treated MCF-10A cells showed no discernable changes in cell cycle phase distribution. Neither diterpenoid affected growth of MDA-MB-231 cells, at the dose range effective for MCF-7 or MCF-10A cells. Ponicidin-treated MCF-7 cells expressed reduced levels of cyclin B1, cdc2, transcription factor E2F, and Rb including phosphorylation at S780. Less pronounced effects were found in cells treated with oridonin. Neither compound altered cyclin D1 and cdk4 in MCF-7 cells. In MCF-10A cells, oridonin was more active than ponicidin in inhibiting the expression of cyclin B1, cdc2, S780-phosphorylated Rb, and E2F. To further investigate induction of apoptosis in MCF-10A cells, we measured changes in NF-κB. Decreases in p65 or p50 forms of NF-κB and its upstream regulator I-κB were found in oridonin-treated MCF-10A and not MCF-7 cells. Taken together, these results provide a mechanistic framework for the cellular effects of ponicidin and oridonin in different stage breast cancer cells.
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Keywords: Oridonin; Ponicidin; Cell cycle control; Induction of apoptosis; Breast carcinogenesis

Breast cancer is a leading cause of morbidity and mortality in women, in developed and increasingly also developing countries [1,2]. In the United States alone, it is estimated that 46,000 women will die of breast cancer and over 2000,000 new cases will be diagnosed each year [3]. In the new millennium, the expected number of annual new cases of breast cancer worldwide could exceed 1.5 million [4]. These statistics emphasize the urgent need for improvements in detection, diagnosis, and treatment of breast cancer.

Recent progress in diagnosis and therapy has increased the survival of women in estrogen-dependent breast cancer. However, the treatment options available for estrogen-independent tumors are far from satisfactory, and consequently carry a poorer prognosis. The intricate details of breast cell growth control have not been completely

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elucidated. Major gaps still exist on the interplay between genetic and endocrine/paracrine/autocrine factors, hormone status and/or changes in hormone receptor functionality/capacity/balance, and dietary influences, which collectively play a major role on the control of mammary cell growth [2,5–7]. Our understanding of the molecular basis of tumorigenesis, control of metastasis, and the mechanism(s) underlying the ability of tumor cells to escape programmed cell death is also incomplete. Not surprisingly, therefore, response of breast carcinoma to various antitumor agents is only modest, particularly those administered as monotherapies [8]. Combination chemotherapies produce more clinically relevant, longer duration, better survival responses than single agent's therapy and hence, have become the standard of management for metastatic, hormone-refractory breast carcinoma, and increasingly, also primary breast carcinoma. They suffer from side effects and propensity to develop chemoresistance [9]. These considerations provide the impetus for individuals diagnosed with breast cancer to explore prevention measures, including complementary and alternative medicine (CAM) such as herbal medicine for a more effective management and possibly cure of their disease. As many as 50% of breast cancer patients use CAM, particularly biopharmacologic and herbal approaches [10]. Herbal medicine is one of the most ancient forms of health care known to humankind and has been used in many cultures throughout history. Typically, herbal medicines emphasize the use of whole extracts from a plant mix or from complex formulations. In many ways, herbal medicine mimics some of the fundamental themes of chemoprevention, a concept originally introduced by Sporn in the 1970s advocating use of natural or synthetic compounds to inhibit or retard cancer development including breast cancer [11–13].

PC-SPES is a multi-component (seven Chinese and one North American) herbal supplement, which has been shown to have potent inhibitory activities against a variety of cancer cells [14]. Initial screen using 95% ethanolic extract of PC-SPES demonstrated that it was most effective in suppressing proliferation and clonogenicity of human MCF-7 breast cancer cells [15]. Since PC-SPES is no longer available commercially, and because its significant anti-tumorigenic activities reportedly may lie in one of its composition herbs, *Rabdosia rubescens* [16,17], we tested the effects of ponicidin and oridonin (Fig. 1), two diterpenoids isolated from *R. rubescens*, in minimally and highly invasive MCF-7 and MDA-MB-231 breast cancer cells [18], for comparison with their normal counterpart MCF-10A. Ponicidin and oridonin showed different effects on the three breast cancer cell lines tested: ponicidin exerted greater inhibition on MCF-7 cells; oridonin showed a more pronounced inhibitory effect in MCF-10A and neither compound demonstrated activities in MDA-MB-231 cells. These results were associated with cell-type dependent targeting at different phases of the cell cycle by either ponicidin or oridonin, concomitant with their effects on expression of cell cycle regulatory proteins. Induction of apoptosis in oridonin-treated MCF-10A may be explained by its ability to significantly suppress the expression of both p65 and p50 forms of NF-κB and its upstream regulator I-κB.

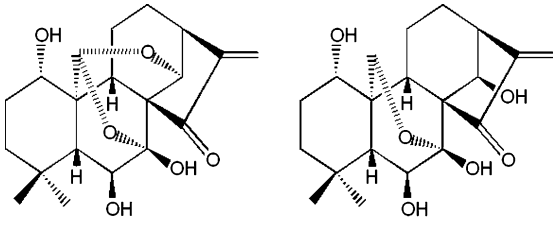


Fig. 1. Structural formulas of ponicidin and oridonin.

Materials and methods

Cell cultures. Breast adenocarcinoma cell lines were purchased from American Type Culture Conditions (ATCC, Rockville, MD) and cultured using the following conditions. MCF-7 cells were maintained in Eagle's minimum essential medium supplemented with 2 mM glutamine and Earle's BSS adjusted to contain 1.5 g/L sodium bicarbonate, 0.1 mM non-essential amino acids, and 1 mM sodium pyruvate, and supplemented with 0.01 mg/ml bovine insulin and 10% fetal bovine serum. MCF-10A cells were maintained in MEGM (mammary epithelial growth medium, serum-free) purchased from Clonetics supplemented with 100 ng/ml cholera toxin. Both MCF-7 and MCF-10A cells were cultured as monolayers in a humidified atmosphere of 5% CO_2 in air. MDA-MB-231 cells were also cultured as monolayers using Leibovitz's L-15 medium supplemented with 2 mM glutamine and 10% FBS, in a CO_2-free, air only tissue culture incubator. Cells were seeded at a density of 5×10^4 cells/ml and passaged by washing the monolayers with phosphate-buffered saline (PBS) followed by a brief incubation with 0.25% trypsin or trypsin/EDTA. The washed cells were isolated by centrifugation and resuspended in culture medium for plating or counting.

Treatment with ponicidin and oridonin. Ponicidin and oridonin were isolated from *R. rubescens*, as described previously [16]. The diterpenoids were dissolved in DMSO as a 2.0 mg/ml stock solution. Each diterpenoid was diluted in culture media to achieve the final concentration indicated for each experiment. Control cells had 0.2% DMSO added to the culture media. In a typical experiment, 10 ml containing 5×10^4 cells/ml was placed in T-75 flasks, together with either diterpenoid or carrier solvent. Cells were harvested at the times specified.

Effect of ponicidin or oridonin on breast cancer cell proliferation and colony formation. Most experiments and results reported in this study were based on 5 μM of either diterpenoid unless otherwise specified. Cells treated for 72 h or as indicated were harvested by trypsinization. Cell number in control and treated cells was counted using a hemocytometer and cell viability was determined by trypan blue dye exclusion [18,19].

The procedure for clonogenicity was performed as described [20]. MCF-7 cells at 200 cells/ml RPMI 1640 and 10% FBS were dispensed into individual wells of a six-well tissue culture dish. Cultures received various doses of ponicidin or oridonin or 0.2% DMSO. After 14 days in culture, the cells were fixed and stained with 0.1% crystal violet to visualize colonies for counting. To test whether the effects of these agents were reversible, some cells were treated with either diterpenoid for 3 days after which the chemicals were removed by extensive washing and incubation was continued for an additional 11 days. The experiments were performed in duplicate or triplicate.

Effect of ponicidin or oridonin on induction of apoptosis and cell cycle progression. Cell cycle phase distribution was analyzed by flow cytometry. Cultures were incubated with 5 μM ponicidin or oridonin for 3

days and harvested. Cells were washed once with PBS and stained with 1.0 μg/ml 4,6-diamidion-2-phenylindole (DAPI; Molecular Probes, Eugene, OR) in a solution containing 100 mM NaCl, 2 mM $MgCl_2$, and 0.1% Triton X-100, pH 6.8, as described [21,22]. The DNA-specific DAPI fluorescence was excited with UV light and collected with appropriate filters in an ICP-22 flow cytometer (Ortho Diagnostic, Westwood, MA). The cell cycle distribution and percentage of apoptotic cells were analyzed by deconvoluting the DNA content frequency histograms with the use of CellFit software (Phoenix Flow, San Diego, CA), as detailed previously [22].

Protein extraction and Western blot analysis. Control and treated cells were rinsed with ice-cold PBS, suspended in buffer (50 μl/10^6 cells) containing 10 mM Hepes, pH 7.5, 90 mM KCl, 1.5 mM $Mg(OAc)_2$, 1 mM dithiothreitol, 0.5% NP40, and 5% glycerol supplemented with 0.5 mM PMSF, 10 μg/ml each of aprotinin, pepstatin, and leupeptin, and lysed by three freeze/thaw cycles [18,19]. The extracts were centrifuged and the clear supernatants were stored in aliquots at −70 °C. Protein concentrations were measured with protein assay reagent (Pierce Chem., Rockford, IL). For Western blot analysis, 10 μg proteins were boiled for 5 min in Laemmli buffer and separated by 10% SDS–polyacrylamide gel electrophoresis (SDS–PAGE). The gels were transferred to nitrocellulose membranes by a semi-dry transfer method. After blocking with buffer containing 5% low-fat milk, the membranes were probed for the expression of cyclins B, D1,and E, cdc2, CDK 4, E2F, Rb, NF-κB p65 and p50, IκB, and β-actin (Santa Cruz Biotechnology, Santa Cruz, CA). Site-specific Rb antibodies (Ser 780 and Thr 821) were purchased from Biosource International (Camarillo, CA). All antibodies used in the experiments were diluted at 1:1000. Specific immunoreactivity was demonstrated by enhanced chemiluminescence (ECL) or color reaction using procedures detailed in the manufacturer's protocol (Kirkegared & Perry Laboratories, Gaithersburg, MD).

Results

Effects of ponicidin and oridonin on colony formation in MCF-7 cells

We first determined the effects of ponicidin and oridonin on colony formation, also referred to as clonogenicity, in MCF-7, MCF-10 A, and MDA-MB-231 cells. This assay measures the ability of tumor cells to grow and form foci, while normal cells become growth contact inhibited. Clonogenicity is therefore an indirect estimation of the propensity of tumor cells to undergo neoplastic transformation. Clonogenicity was determined by plating a fixed number of MCF-7 cells onto multiple well tissue culture dishes, with and without addition of varying concentrations of oridonin and ponicidin. Cells with and without treatment were maintained in culture for an additional 14 days to allow formation of colonies. Size and number of colonies were visually inspected by fixing and staining in 0.1% crystal violet. Fig. 2A and B shows that clonogenicity of MCF-7 cells was significantly reduced by incubation with ponicidin or oridonin, with the former showing a much more pronounced inhibition, already evident at $\geqslant 0.5$ μM whereas oridonin's inhibitory effect only became visually apparent between 1.0 and 5.0 μM. Notably also, not only did ponicidin reduce the number of colonies formed, it visibly decreased the size of the colonies as well. A 3-day exposure followed by removal of the test compound produced almost identical results, suggesting that the effects of either diterpenoid were largely irreversible. As expected, normal

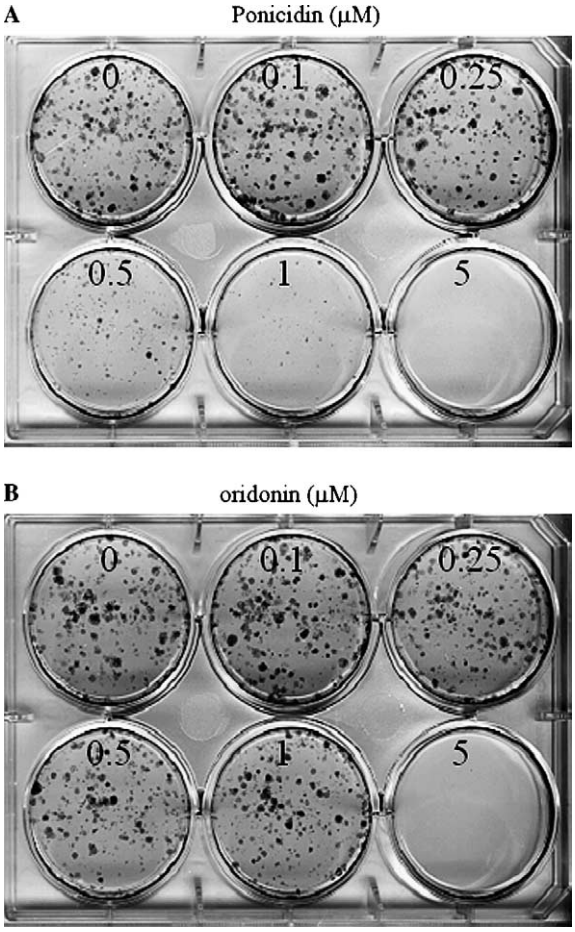


Fig. 2. Effects of oridonin or ponicidin on clonogenicity in MCF-7 cells. Clonogenicity was assayed as detailed in Materials and methods.

MCF-10A cells did not form colonies. Contrary to expectation, MDA-MB-231 cells also had poor ability to form colonies (data not shown).

Effects of ponicidin and oridonin on growth of MCF-7, MCF-10A, and MDA-MB-231 breast cancer cells

We also tested the effects of ponicidin and oridonin on growth of MCF-7 cells. For comparison, effects on MCF-10A and MDA-MB-231 cells were also studied. We first determined that, in the absence of treatment by chemicals, these breast cell lines had comparable net increases in cell number over the duration of the experiment (data not shown). When the breast cells were treated with various concentrations of either chemical, results in Figs. 3A–C were observed. A 72-h treatment of MCF-7 cells with ponicidin or oridonin resulted in a comparable reduction in growth (Fig. 3A). In contrast, MCF-10A cells showed a more pronounced growth inhibition by oridonin, compared to ponicidin (Fig. 2B). Interestingly, MDA-MB-231 showed little, if any, inhibitory response to either diterpenoid. In time course experiments, we demonstrated that the observed inhibition of cell growth in MCF-7 or MCF-10A cells by either diterpenoid, to a large extent, was due to cellular cytostasis (data not shown).

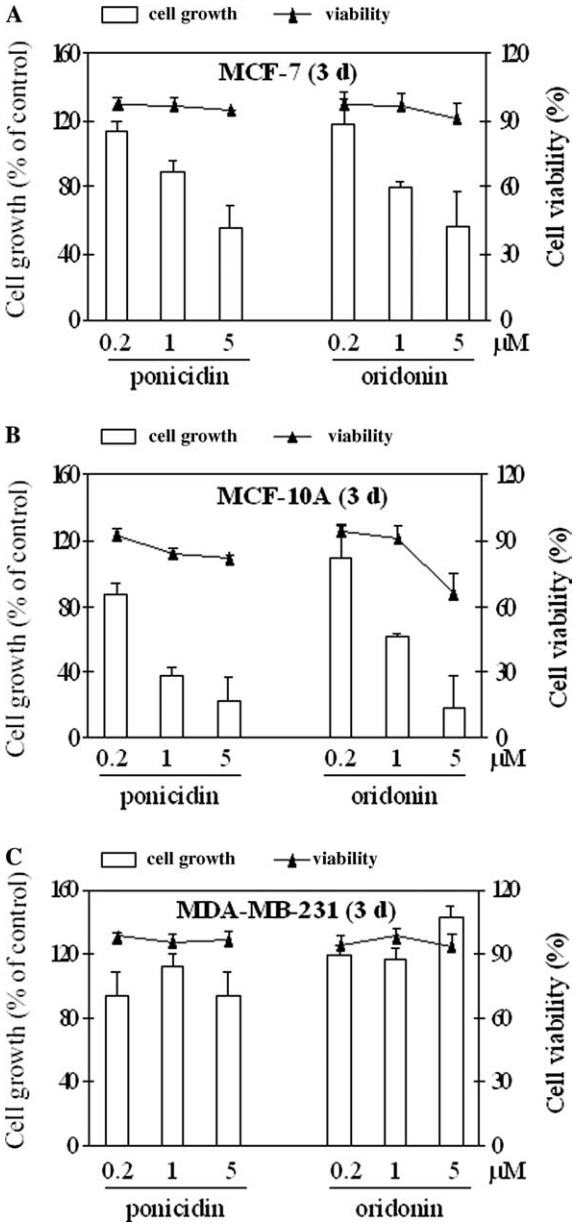


Fig. 3. Effects of diterpenoids on growth and cell viability of MCF-7 (A), MCF-10A (B), and MDA-MB-231 (C) cells.

To explore the underlying basis for the antiproliferative activities of ponicidin and oridonin, cell cycle analyses were performed, in the three cell types, with and without addition of 5 μM oridonin or ponicidin for 3 days. Cell cycle phase distribution was analyzed by flow cytometry. Control and treated cultures were harvested, washed with PBS and stained with 1.0 μg/ml DAPI [21,22], and analyzed as detailed in Materials and methods. Results in Fig. 4A show that ponicidin affected S/G_2M, correspondingly reducing and increasing cells in G_1 and an S phase, in MCF-7 cells. By comparison, oridonin caused a 12.8% increase in the G_1 phase and a corresponding decrease in S and G_2M phases in the same cell type (Fig. 4A). Similar cell cycle analysis in MCF-10A cells showed little effect on cell cycle phase distribution in cells treated with ponicidin; rather, ponicidin resulted in induction of apoptosis as demonstrated by appearance of cells with fractional DNA content (Fig. 4B). The ability to induce apoptosis was even more evident in oridonin-treated MCF-10A cells. Additionally, oridonin also blocked progression of MCF-10A cells from S to G_2/M phase (Fig. 4B). As expected, ponicidin had no effect on cell cycle phase distribution in MDA-MB-231 cells. Surprisingly, oridonin suppressed MDA-MB-231 cell cycle progression at the G_1 phase (Fig. 4C), similar to what was observed in MCF-7 cells (Fig. 4A).

Modulation of cell growth regulatory protein expression by ponicidin and oridonin in MCF-7 and MCF-10A breast cancer cells

Further evidence that these two diterpenoids elicited different cell cycle effects in MCF-7 and MCF-10A breast cancer cells came from analysis of changes in cell cycle regulatory protein expression in control and treated cells. For instance, ponicidin significantly reduced expression of cyclin B1 in MCF-7 cells, while oridonin had a more pronounced effect in reducing cyclin B1 expression in MCF-10A cells (Fig. 5). Moreover, cdc2 levels were also substantially affected by oridonin in MCF-10A levels, whereas neither diterpenoid had much effect on cdc2 in MCF-7 cells (Fig. 5). We also analyzed for changes in cyclin D1, cyclin E, and cdk4 in both cell lines, in response to treatment by either ponicidin or oridonin. No significant change in their expression by either diterpenoid was observed (Fig. 5).

The changes in cyclin B1 and cdc2 may explain in part the observed effect of ponicidin on suppression of S/G_2M in MCF-7 cells but cannot easily support the G_1/S cell cycle effects of oridonin in MCF-10A cells. Accordingly, we further analyzed for changes in the retinoblastoma tumor suppressor protein Rb, whose expression and state of phosphorylation play a pivotal role in the control of G_1/S cell cycle checkpoint [23,24]. Notably, hypophosphorylated Rb is found in the noncycling G_0 and early G_1 cells, whereas hyperphosphorylated Rb is characteristic of proliferating cells in the S and G_2/M phases. The status of Rb phosphorylation controls the availability of transcription factor E2F responsible for growth control. Western blot analysis demonstrated that treatment with 5 μM oridonin or ponicidin significantly reduced both hyperphosphorylated and unphosphorylated Rb levels, in MCF-10A cells, whereas only minor changes were found in MCF-7 cells (Fig. 5). In addition to the changes in Rb phosphorylation, downregulation of E2F by either diterpenoid was also observed (Fig. 5). These results imply that one effect of these diterpenoids in MCF-10A cells was to increase the proportion of unphosphorylated Rb and, as a consequence, an increase in binding capacity for transcription factor E2F, which might contribute to the observed suppression of G_1/S transition (Fig. 4). Although neither diterpenoid affected cyclin D1/CDK 4 expression, a reduction in Rb phosphorylation at S780, purportedly targeted by cyclin

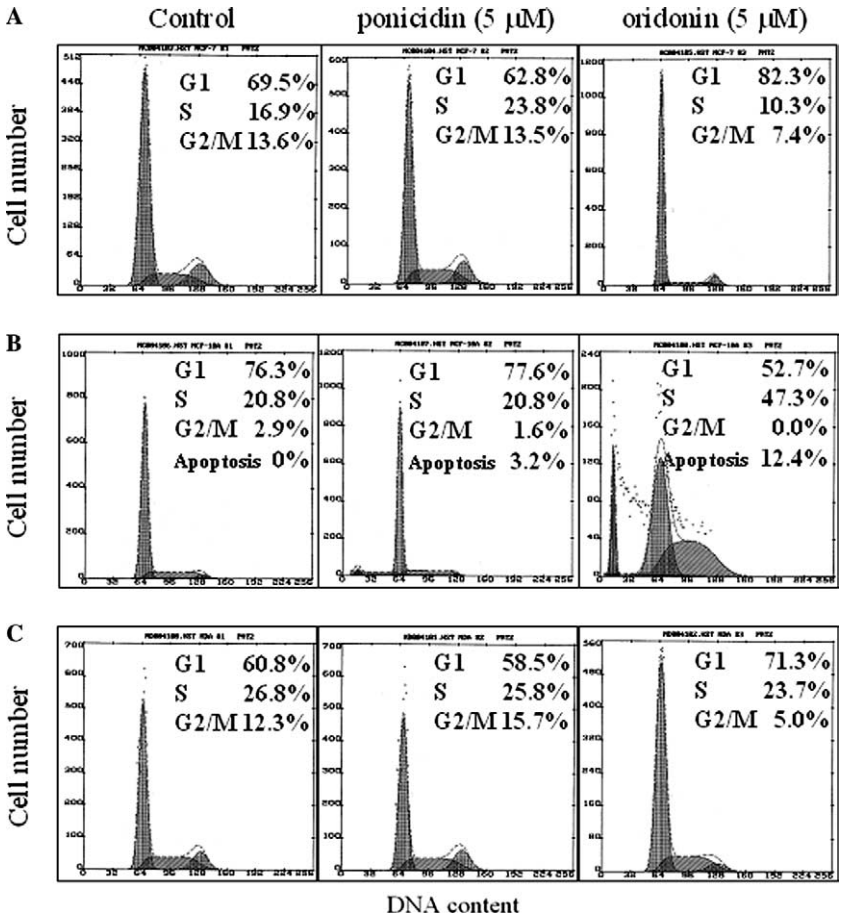


Fig. 4. Cellular DNA content frequency histograms showing the cell cycle phase distribution and apoptosis of breast cells following 3-day (A–C) treatment with 5 μM ponicidin or oridonin. (A) MCF-7 cells. (B) MCF-10A cells. (C) MDA-MB-231 cells. Flow cytometric analysis was performed as described in Materials and methods. Note distinct accumulation of cells in G_2/M and modest proportion of cells with fractional DNA content (sub-G_1 cell population; typical of apoptosis) in cultures treated with either diterpenoid.

D1 and CDK4, was found in MCF-10A cells treated with ponicidin or oridonin (Fig. 5).

Effect of ponicidin and oridonin on expression of NF-κB

Since MCF-10A cells also showed induction of apoptosis, compared to MCF-7 cells, and because transcription factor NF-κB is known to be functionally associated with cell survival [25,26], we measured changes in the steady state level of NF-κB in MCF-7 and MCF-10A cells treated with either diterpenoid. Pronounced decreases in p65 and p50 forms of NF-κB and its upstream regulator I-κB were found in oridonin-treated MCF-10A cells, whereas little to no change in these biochemical parameters was found in MCF-7 cells (Fig. 6).

Discussion

Rabdosia rubescens is one of several Chinese herbs present in the once popular dietary supplement, denoted PC-SPES. Use of the entire plant of this Chinese herb purportedly shows digestive benefit systems and anecdotal evidence of efficacy for esophageal carcinoma, with little toxicity [27]. The main active components of *R. rubescens* are tetracyclic diterpenoid compounds, such as oridonin and ponicidin [28]. More recently, one of its active constituents, oridonin has been evaluated as adjunct to standard cisplain therapy, in tissue culture and in laboratory animal studies [29]. The possibility that *R. rubescens* may have efficacy against breast cancer came from initial screen showing that human MCF-7 breast carcinoma cells was highly sensitive to 95% ethanolic extract of PC-SPES, in comparison to various other malignant human cell lines [15]. More recently, 70% ethanolic extracts of *R. rubescens* were shown to strongly inhibit proliferation of MDA-MB-231 cells, accompanied by marked up-regulated induction of apoptosis [17]. Oridonin from *R. rubescens* was found to induce growth inhibition and proliferation in a variety of human cell lines [30–36] and to enhance phagocytosis of apoptotic bodies [37,38]. To our knowledge, a systematic comparison of the effects of different diterpenoids from *R. rubescens* in breast cancer lines with varying invasiveness has not been performed. Therefore, we tested in the present studies the effects of these two diterpenoids in MCF-7 and MDA-MB-231 cells, as representing low and high invasive attributes of breast

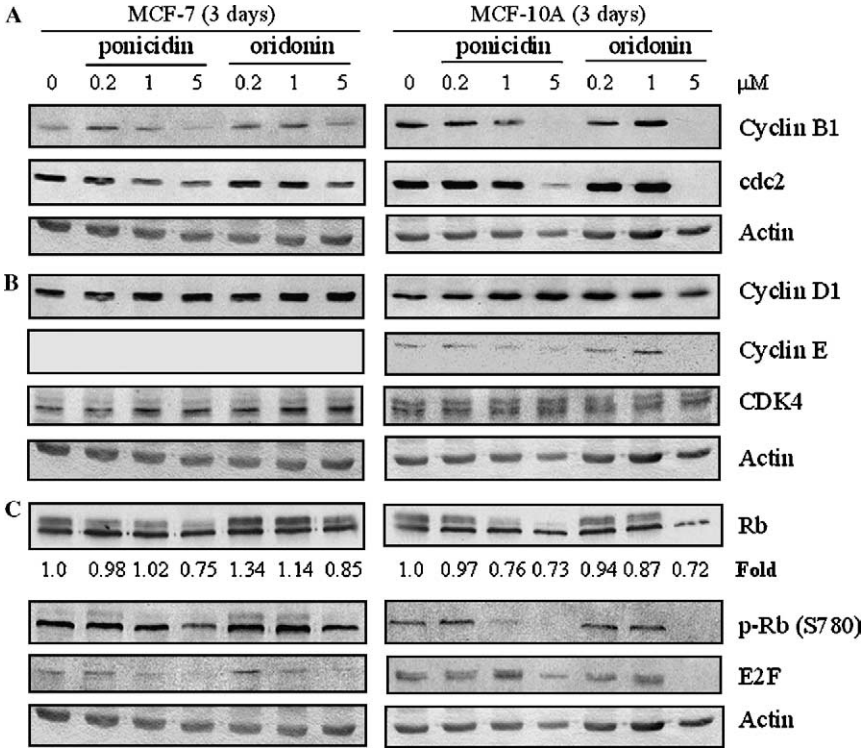


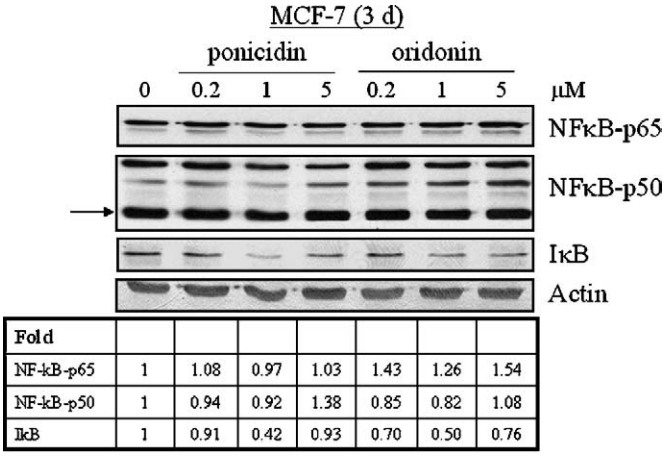
Fig. 5. Changes in cell cycle regulatory proteins in control and 3 day 5 μM diterpenoid-treated breast cancer cells. Expression of cyclin B1, cdc2, and actin (A), cyclin D1, cyclin E, CDK4, and actin (B), and Rb, p-Rb (S780), E2F, and actin (C), respectively, was determined by immunoblot analysis as detailed in Materials and methods.

carcinoma, for comparison with their normal counterpart, MCF-10A cells.

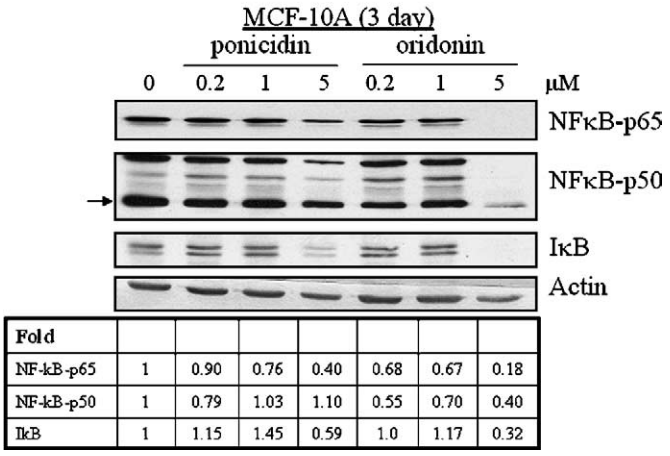
In agreement with previous reports, we observed that proliferation in MCF-7 cells was inhibited to a comparable degree by oridonin and ponicidin (Fig. 3A). Clonogenicity assay using these cells, however, showed ponicidin to be more effective than oridonin (Fig. 2). Flow cytometric analysis revealed that 5 μM ponicidin induced S/G_2M arrest while oridonin elicited a G_1/S block in MCF-7 cells (Fig. 4A). Little apoptosis was shown in MCF-7 cells treated with either diterpenoid (Fig. 4A). To investigate the biochemical basis for the observed flow cytometric results, we analyzed changes in cell cycle regulatory proteins. Ponicidin-treated MCF-7 cells showed diminished expression of cyclin B1 and cdc2, reduced phosphorylation of Rb including S780, and also lowered levels of transcription factor E2F, to a more significant degree than cells treated with an identical concentration of oridonin. These results contrasted with little to no observed changes in cyclin D1 and CDK4 expression in MCF-7 cells treated with either compound (Fig. 5).

Proliferation of MCF-10A cells was markedly suppressed by oridonin and to a lesser extent by ponicidin (Fig. 3B). Similarly, treatment with oridonin resulted in marked induction of apoptosis, as well as inhibition of cell cycle progression (Fig. 4B). Notably, MCF-10A cells treated with oridonin showed accumulation of cells in the S phase; virtually little to no cells were detectable in the G_2M phase, concomitant with a marked increase in apoptosis. In contrast, no changes in cell cycle phase distribution were observed in ponicidin-treated MCF-10A cells; instead, induction of apoptosis was clearly evident, compared to untreated cells. Measurement of the same cell cycle parameters in MCF-10A cells showed that oridonin had a more pronounced effect in inhibiting the expression of cyclin B1, cdc2, phosphorylation of Rb, E2F, in comparison with cells treated with ponicidin (Fig. 5).

Since MCF-10A cells also showed substantial induction of apoptosis, compared to MCF-7 cells, and because transcription factor NF-κB is known to be functionally associated with cell survival, we measured changes in the steady-state level of NF-κB in MCF-7 and MCF-10A cells treated with either diterpenoid. Decreases in either p65 or p50 forms of NF-κB and its upstream regulator I-κB were more pronounced in oridonin-treated MCF-10A cells than in ponicidin-treated cells (Fig. 6). These results agreed with the extent of apoptosis induced by these diterpenoids. In contrast, little to no change in these biochemical parameters was found in MCF-7 cells (Fig. 6). In addition to providing a plausible mechanistic explanation for the effects of oridonin and ponicidin in different stage breast cancer cells, these results may have other significance as well. NF-κB is one of the several transcription factors involved in the production of IL-6 [26]. IL-6 is a susceptibility factor that determines racial and/or ethnic differences in breast cancer survival [39]. Suppression of NF-κB may also result in the amelioration of cachexia, as has been previously suggested [40].



Fold							
NF-kB-p65	1	1.08	0.97	1.03	1.43	1.26	1.54
NF-kB-p50	1	0.94	0.92	1.38	0.85	0.82	1.08
IkB	1	0.91	0.42	0.93	0.70	0.50	0.76



Fold							
NF-kB-p65	1	0.90	0.76	0.40	0.68	0.67	0.18
NF-kB-p50	1	0.79	1.03	1.10	0.55	0.70	0.40
IkB	1	1.15	1.45	0.59	1.0	1.17	0.32

Fig. 6. Effect of treatment by ponicidin or oridonin on changes in expression of NF-κB in breast cancer cells. Control and treated cells were harvested and total protein extracts were prepared, separated by SDS–PAGE and analyzed for expression of NF-κB p65, p50 and I-κB by Western blot analysis.

Surprisingly, neither diterpenoids had an effect on growth of MDA-MB-231 cells at the same concentrations used to treat the MCF-7 or MCF-10A cells (Fig. 3C). Thus, it seems likely that other specific diterpenoids, different from oridonin or ponicidin, or interaction among diterpenoids could account for the strong inhibition of proliferation and induction of apoptosis in MDA-MB-231 cells treated with 70% ethanolic extracts of *R. rubescens* [17].

Oridonin has been reported to dose-dependently induce spectral shifts and an increase in absorption band intensity when incubated with double-stranded calf thymus DNA [32]. Using electrophoretic mobility shift assays, Leung et al. [27] provided evidence that oridonin and ponicidin directly interfere with the DNA-binding activity of NF-κB to its response DNA sequences, possibly through binding to the cysteine binding domains of p65 or p50 and hence modifying the transcriptional competence of NF-κB. Conceivably, this ability to interfere with the transcriptional engagement of NF-κB by the diterpenoids could facilitate a more rapid turnover of p65 and p50 forms of NF-κB in MCF-10A cells. Because neither p65 or p50 forms of NF-κB were affected by oridonin or ponicidin in MCF-7 cells, it is possible that they are either modified or in a state inaccessible to either oridonin or ponicidin. These remain possibilities that await further investigation and are currently under consideration in our laboratory.

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