Apigenin Acts on the Tumor Cell Invasion Process and Regulates Protease Production

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Abstract: Apigenin is a widely distributed plant flavonoid and was proposed as an antitumor agent. In this study, we investigated the apigenin effects on the protease-mediated invasiveness in an estrogen-insensitive breast tumor cell line MDA-MB231. The results show that apigenin at 22.8–45.5 \(\mu\)M (2.5–10 \(\mu\)g/ml) strongly inhibited, in a dose-dependent manner, tumor cell invasion through Matrigel, cell migration, and cell proliferation. We show that apigenin treatment from 22.8 \(\mu\)M (2.5 \(\mu\)g/ml) led to a partial decrease in urokinase-plasminogen activator expression and to a total inhibition of phorbol 12-myristate 13-acetate-induced matrix metalloproteinase-9 secretion. We also demonstrate in the apigenin-treated cells a defective adhesion to Matrigel and a G2-M cell cycle arrest. Taken together, our results demonstrate that apigenin is a pleiotropic effector affecting protease-dependent invasiveness and associated processes and proliferation of tumor cells.

Introduction

The long-known preventive effect of plant-based diets on tumorigenesis and other chronic diseases is well documented (1). Several cancers, including breast cancer, are among those that have a lower incidence in Asia than in Western countries (2). These observations have been attributed to the Asian dietary regimen, rich in flavonoid-containing plants, which are thought to be antitumorigenic. The flavonoid family is a group of common phenolic plant pigments, present in significant amounts in various plants like soya, fruits, and vegetables, as well as in tea, coffee, and wine (3). Yet the molecular mechanisms involved in the action of these flavonoids are so far not well documented.

Among the flavonoids, apigenin is one of the most effective in inducing cell growth inhibition and is relatively nontoxic and nonmutagenic (4,5). It has been reported that apigenin has chemopreventive effects (6,7), and it was suggested to involve the p53-p21/waf1 response pathway (8). Apigenin arrests rat neuronal cells at the G2-M phase and promotes their differentiation (3). Moreover, it has antiproliferative effects on various cell lines (10), including breast cancer cells (5). Two cell cycle regulators, p34 (cdc2) kinase and cyclin B1, were shown to be decreased in colon carcinoma cell lines after apigenin treatment, resulting in growth inhibition and reversible arrest of the G2-M phase (11). In HL-60 leukemic cells, treatment with apigenin (60 \(\mu\)M) induced apoptosis and caused a rapid induction of caspase-3 activity, elevation of reactive oxygen species production, release of mitochondrial cytochrome c into the cytosol, and subsequent induction of pro-caspase-9 processing (12). Recently, apigenin was also shown to inhibit melanoma B16-BL6 growth and metastatic potential in vivo (13).

It has been suggested that apigenin affects cells by interfering with nuclear factor (NF)-\(\kappa\)B transcriptional activation (14), which regulates the expression of several genes involved in cellular adhesion and invasion, such as intercellular adhesion molecule 1, ELAM-1, vascular cell adhesion molecule 1 (14), and also type IV collagenases [matrix metalloproteinase (MMP)-2 and MMP-9] (15,16). Recently, it has been shown that apigenin inhibited mitogen-activated protein kinase (MAPK) activity and its downstream oncogene expression, such as c-jun and c-fos (17). In another study, the inhibitory effect of apigenin on proliferation of thyroid carcinoma cells was associated with an inhibition of both epidermal growth factor (EGF)-receptor tyrosine autophosphorylation and phosphorylation of its downstream effector MAPK (18). MAPKs are thought to integrate a variety of mitogenic signals initiated by various cell surface receptors and regulate transcription of many genes as intermediates in the signal transduction pathway (19,20). Constitutively active MAPKs are associated with carcinogenesis and metastasis in human breast cancer (21).

This study investigated the effect of apigenin on cell invasion. Cell invasion involves a series of proteolytic events involving proteolytic enzymes such as urokinase-plasminogen activator (u-PA) and MMPs. The concept that these proteases are involved in invasion and metastasis, as well as tumor angiogenesis, has been well accepted (22). Urokinase...
is a serine protease, and expressions of u-PA and its cell surface receptor u-PAR are considered to be poor prognostic indexes of breast cancer (23,24). Collagenase type IV and MMP-9, in particular, have also been shown to play a critical role in tumor invasion and metastasis (25,26). Considering that MAPKs have been reported to regulate the expression of MMP-9 in breast epithelial cells (27) and that gene expression of proteases, in particular u-PA and MMP-9, is under the regulation of NF-κB (16,28), we postulated that apigenin would affect cell invasion by regulating protease production. To test this hypothesis, we investigated the effect of apigenin on an estrogen-negative breast tumor cell line (MDA-MB231), which was highly proliferative and invasive.

Our results show that the adhesive and invasive behaviors of apigenin-treated cells were strongly inhibited. Exposure of MDA-MB231 cells to apigenin blocked the constitutive expression of u-PA as well as the phorbol 12-myristate 13-acetate (PMA)-induced MMP-9 secretion. We also show that cell migration and cell proliferation were affected by apigenin, thus demonstrating that apigenin is a pleiotropic effector on tumor cells.

Material and Methods

Material and Chemicals

All culture products were obtained from GIBCO, plastics from Costar, monoclonal antibodies against u-PA (no. 3689) and u-PAR (no. 3936) from American Diagnostica (Greenwich, CT), Annexin V-fluorescein isothiocyanate kit from Bioproducts (Boehringer Ingelheim, Heidelberg, Germany), and apigenin, dimethyl sulfoxide (DMSO), and bovine serum albumin (BSA) from Sigma. Apigenin was dissolved in DMSO and prepared in 90 mM (10 mg/ml) stock solution. Matrigel was obtained from Becton-Dickinson (Bedford, MA), recombinant single-chain urokinase (scu-PA) from Grunenthal (Aachen, Germany), and recombinant human basic fibroblast growth factor (bFGF) from R & D Systems (Abingdon, UK).

Cell Culture and Treatment of Cells

The human breast adenocarcinoma cell line MDA-MB231 was routinely cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 2 mM glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin, and 10% fetal calf serum (FCS). For experiments, subconfluent cells were washed and incubated with various concentrations of apigenin, ranging from 0 to 180 μM (0–20 μg/ml), in serum-free medium for 24 hours. The concentration of vehicle (DMSO) was adjusted to 0.2% (vol/vol) in all experiments, including control. Trypan blue exclusion test revealed that after 48 hours of incubation >95% of the cells were viable in the control experiments, while in the presence of apigenin the viability was ~90% at the highest concentra-

Quantification and Activity of u-PA: Enzyme-Linked Immunosorbent Assay and Zymogram Assays

Cells were incubated with the indicated concentrations of apigenin in serum-free medium for 24 hours. Culture media were then collected and centrifuged before use. The cells were harvested by short exposure to EDTA and exposed or to scu-PA. Volumes of culture media were adjusted to the same number of cells. The u-PA secreted into serum-free media was determined by indirect enzyme-linked immunosorbent assay (IMUBIND u-PA ELISA kit) according to the manufacturer’s instructions (American Diagnostica). Zymography was used to quantify the cell-bound and the free u-PA activity. It was performed with casein gel containing plasminogen, as described elsewhere (29). A volume of 5 μl of washed cells (25 × 10^5) or culture medium was laid down onto the gels. The gels were observed under dark-field illumination for the areas of lysis after a five-hour incubation at 37°C. Amiloride (Sigma) at 1 mM was included in a control gel to inhibit u-PA activity to ensure the specificity of u-PA-dependent caseinolysis.

Flow Cytometric Analysis for u-PA, u-PAR, and DNA Content

To examine the cell surface expression of u-PA or u-PAR, cells were treated with apigenin for 24 hours. The washed and resuspended cells were incubated with 10 μg/ml of a monoclonal antibody directed against u-PA or u-PAR for 30 minutes at 4°C. We used a fluorescein isothiocyanate-conjugated anti-mouse rabbit immunoglobulin G (BioSystem, Compiègne, France) to reveal positive cells. After they were washed, the cells were fixed with 4% paraformaldehyde and analyzed in a flow cytometer FACScan (Becton-Dickinson) with the CellQuest software.

For cell cycle analysis, cells were treated with various concentrations of apigenin for 48 hours in serum-free medium. The cell suspension was washed and incubated with propidium iodide. After 10 minutes of incubation, the cell suspension was directly analyzed on a FACScan.

Quantification by Reverse Transcription-Polymerase Chain Reaction

Subconfluent cells were cultured for 24 hours in serum-free medium with various concentrations of apigenin. The cells were harvested by short exposure to trypsin-EDTA and pelleted. The mRNA were extracted with the SV Total RNA
Isolation System (Promega, Madison, WI) according to the manufacturer’s recommendations. The Promega access reverse transcription-polymerase chain reaction (RT-PCR) system was used in this assay. Sense primer (5’-GGCAGCAATGAACTTCAATCGTCC-3’) and antisense primer (5’-TATTTCCAGGTGACGCGGCCTCC-3’) were used for the detection of u-PA mRNA, 5’-CTGGA-GCTGAGGAAAG-3’ and 5’-CATGCTGATGAGCCACAGG-3’ for u-PAR mRNA, and 5’-ATCTGCG-ACCACACCTTCTACAATGAGCTGCG-3’ and 5’-CGTCACTACTCTGCTGTAGCACACATCTGC-3’ for β-actin mRNA as control. Total RNA (0.5 µg) and 50 pmol of each primer were added to the RT-PCR. The RT was performed at 48°C for 50 minutes followed by a cycle reaction of PCR at 94°C (2 min), 60°C (30 s), and 68°C (1 min), then 29 cycles at 94°C (1 min), 60°C (30 s), and 68°C (1 min), and finally 68°C (7 min). The RT-PCR products were observed on 2% agarose gels.

Analysis of MMP-9 Activity by Zymography

Detection of MMP-9 secretion to the conditioned media was determined by zymography as described elsewhere [30]. Briefly, sample volumes were adjusted to the number of cells and loaded on 10% SDS-polyacrylamide gels copolymerized with 1 mg/ml gelatin. After electrophoresis, the gels were extensively washed with 2.5% Triton X-100, incubated overnight at 37°C in appropriate enzyme buffer (50 mM Tris, 2 mM CaCl2, 0.02% NaN3, pH 7.5), and then stained with 0.1% Coomassie brilliant blue. The MMP-9 activity was visualized as transparent bands against a blue background.

Cell Invasion and Migration Assay

The invasion assay was performed essentially as described previously [31]. Briefly, 6.5-mm-diameter polycarbonate filters of 8-µm pore size (Transwell, Costar, Badhoevedorp, The Netherlands) were coated with 20 µg of basement membrane Matrigel, dried, and reconstituted at 37°C with DMEM before use. The lower chambers were filled with conditioned medium of fibroblasts as chemoattractant, supplemented with 2 mg/ml BSA and 10 ng/ml bFGF. After a brief exposure to EDTA, the cells were collected and then added on the upper chamber (10⁶ cells/chamber in DMEM containing 0.2 mg/ml BSA and 10% FCS). Apigenin or DMSO (control) was also added to the upper chamber. After 24 hours of incubation at 37°C, the filters were removed and stained with a Diff-Quick kit (DADE). The number of cells that had spread on the lower surface of the filter was counted with a microscope. The migration assay was described as for the invasion study, except the polycarbonate filters were not coated with Matrigel and migration time was limited to five hours. Each invasion or migration experiment was carried out in duplicate and repeated at least twice.

Cell Adhesion

Adhesion assays of tumor cells to basement membrane were performed in 96-wells plates (Costar) using substrate for cell endogenous phosphatases as previously described [32]. The wells were coated overnight at 4°C with Matrigel (20 µg/100 µl) or 1% BSA as control. Nonspecific binding sites were blocked with 1% BSA for one hour at 37°C and washed three times with adhesion buffer (10 mM HEPES, 140 mM NaCl, 5.6 mM glucose, 5.4 mM KCl, 2 mM CaCl2, 1 mM MgCl2, 1 mM MnCl2, pH 7.4). The cells (5 × 10⁴/well in adhesion buffer), pretreated or not by apigenin, were allowed to adhere for 20 minutes at 37°C. Then, nonadhered cells were removed by washing three times with adhesion buffer. The substrate for phosphatases (p-nitrophenyl phosphate, Sigma) in acetate buffer, 0.1% Triton X-100, pH 5.5, was added to each well for one hour of incubation at 37°C. The reaction was stopped with 1 N NaOH, and absorbance was measured at 405 nm. Each point was determined in triplicate.

Cell Proliferation Assay

Stock cultures were detached by trypsin, and cells were seeded in 96-wells plates at 1.2 × 10⁴ cells/well. Cells were grown for 24 hours with 10% FCS culture medium, then further cultured for 30 hours in fresh medium with 10% FCS in the presence of 0.2% DMSO (control) or various concentrations of apigenin. Then, 1 µCi of [³H]thymidine (Amersham, Courtaboeuf, France) was added to each well, and the cells were incubated for 18 hours. The cells were extensively washed, and cell-incorporated radioactivity was counted with a beta counter. Each condition was run in triplicate.

Statistical Analysis

All experimental results were analyzed with one-way analysis of variance using Microsoft Excel (Microsoft, Redmond, WA).

Results

Apigenin Regulates Protease Production

Inasmuch as cancer invasiveness is a complex mechanism involving pericellular proteolytic activity, we first examined the regulation by apigenin of the production of u-PA and MMP-9. The u-PA secretion into the culture media was inhibited by apigenin in a dose-dependent manner as demonstrated by enzyme-linked immunosorbent assay measurements (Figure 1A). Cell surface-bound u-PA, analyzed by flow cytometry, was also decreased in a dose-dependent manner after apigenin treatment (Figure 1B, a–d). In contrast, constitutive u-PAR expression was not affected by apigenin (Figure 1B, e). The decrease in u-PA expression and the unaltered u-PAR expression were confirmed by
zymography and RT-PCR. When the culture media or the living cells were layered onto a plasminogen-rich casein gel, a clear decrease in secreted and membrane-bound u-PA was observed in apigenin-treated cells (Figure 1C). Gel lysis was totally inhibited in the presence of amiloride (not shown), demonstrating that the lysis was u-PA specific. However, the expression of u-PAR and its ability to bind u-PA were not altered in these cells, inasmuch as the effect of apigenin was reversed by preincubation of the treated cells with recombinant single-chain u-PA (scu-PA) to saturate u-PA binding sites at cell surface before deposit on gel. D: agarose gel electrophoresis of reverse transcription-polymerase chain reaction products of u-PA and u-PAR after mRNA isolation from control cells treated with dimethyl sulfoxide (1) and cells treated for 24 h with 2.5 (2), 5 (3) and 10 (4) µg/ml (0, 22.8, 45.5, and 90 µM) apigenin. E: zymography analysis of MMP-9 secretion on media cultured for 24 h with phorbol 12-myristate 13-acetate (PMA). Volumes loaded were adjusted to number of cells. In C and E, typical results are shown for ≥4 repeated experiments.

Figure 1. Effects of apigenin on urokinase-plasminogen activator (u-PA) and u-PA receptor (u-PAR) expression and on matrix metalloproteinase-9 (MMP-9) expression. A: quantification by enzyme-linked immunosorbent assay of u-PA secreted by MDA-MB231 cells during 24 h of incubation with apigenin. Values are means ± SD of duplicate results from 3 experiments. B: analysis by flow cytometry of MDA-MB231 cells pretreated for 24 h with apigenin at 2.5 (a), 5 (b), 10 (c), and 20 (d) µg/ml (22.8, 45.5, 90, and 180 µM) for cell surface expression of u-PA and typical profile for cell-surface expression of u-PAR at 2.5–20 µg/ml (22.8–180 µM) apigenin (e). Values are a representative example of 3 independent experiments. C: inhibition by apigenin of u-PA secretion into medium and of cell surface u-PA binding as demonstrated by zymography. For 1 set of experiments, cells were incubated with recombinant single-chain u-PA (scu-PA) to saturate u-PA binding sites at cell surface before deposit on gel. D: agarose gel electrophoresis of reverse transcription-polymerase chain reaction products of u-PA and u-PAR after mRNA isolation from control cells treated with dimethyl sulfoxide (1) and cells treated for 24 h with 2.5 (2), 5 (3) and 10 (4) µg/ml (0, 22.8, 45.5, and 90 µM) apigenin. E: zymography analysis of MMP-9 secretion on media cultured for 24 h with phorbol 12-myristate 13-acetate (PMA). Volumes loaded were adjusted to number of cells. In C and E, typical results are shown for ≥4 repeated experiments.

zymography and RT-PCR. When the culture media or the living cells were layered onto a plasminogen-rich casein gel, a clear decrease in secreted and membrane-bound u-PA was observed in apigenin-treated cells (Figure 1C). Gel lysis was totally inhibited in the presence of amiloride (not shown), demonstrating that the lysis was u-PA specific. However, the expression of u-PAR and its ability to bind u-PA were not altered in these cells, inasmuch as the effect of apigenin was reversed by preincubation of the treated cells with recombinant scu-PA (Figure 1C). In addition, the RT-PCR results show that the mRNA level of u-PA was downregulated by apigenin, although only at 90 µM (10 µg/ml), while the mRNA level of u-PAR was not modified (Figure 1D). It appeared therefore that reduced u-PA expression was correlated with a reduced cell-bound u-PA activity.

As shown by gelatin zymography, MDA-MB231 cells constitutively secreted MMP-9, which was highly stimulated by the tumor promoter PMA (Figure 1E). Only the proenzyme form of MMP-9, with molecular mass of 92 kDa, could be detected in the conditioned media. The addition of apigenin did not significantly affect the basal secretion of MMP-9 but completely abolished PMA-induced MMP-9 secretion (Figure 1E).

Apigenin Affects Cell Migration and Invasion Capacities

We next examined the effect of apigenin on MDA-MB231 cell invasion through Matrigel, a reconstituted extracellular matrix (ECM). As is shown in Figure 2A, 45.5 µM (5 µg/ml) apigenin greatly reduced the invasiveness of these cells: 74% inhibition compared with control. When tumor cells were saturated with exogenous scu-PA, the invasion was increased in apigenin-treated and untreated cells,
although invasion was still reduced in the presence of apigenin. We have also shown that the apigenin-treated cells have a reduced motility, as demonstrated in a Matrigel-free migration system. The migration was inhibited by ~15% at 22.8 μM (2.5 μg/ml) and 45% at 45.5 μM (5 μg/ml), as shown in Figure 2B. As for invasion, preincubation with scu-PA stimulated cell migration in apigenin-treated and untreated cells. However, the inhibition by apigenin was still evident at the same proportions as without scu-PA (Figure 2B).
Apigenin Affects MDA-MB231 Cell Morphology

The MDA-MB231 cells were exposed to various concentrations (0–180 μM, i.e., 0–20 μg/ml) of apigenin, and the cell morphology was observed after 24 hours of treatment in a serum-free medium (Figure 5). Morphological changes were clearly observed at 45.5 μM (5 μg/ml) apigenin and became very pronounced at 90 and 180 μM (10 and 20 μg/ml), since DMSO alone has no effect. The cells lost the smooth surface boundary, became irregular, and developed dendrite-like structures with extended arborization. The morphological changes were reversed on apigenin removal. Inasmuch as a role for MAPKs in the initiation of microtubular reorganization has been demonstrated (33), such morphological changes in treated cells could be related to the apigenin MAPK inhibitor activity.

Discussion

The preventive effect of flavonoids on cancer incidence makes them attractive candidates for the development of anticancer agents. Apigenin, one of flavonoids, has been described as a chemopreventive compound (6,7) and as an inhibitor of certain signal transduction pathways (14,17). We demonstrated here that apigenin affects proteolytic activity and invasion capacity of breast tumor cells in addition to other reported aspects, such as inhibition of cell proliferation.

First, apigenin treatment caused a remarkable decrease at the mRNA and protein levels in the expression of u-PA, a well-recognized activator of pericellular proteolysis in migrating cells (34). In particular, we show an important decrease in cell membrane-bound u-PA activity. Because the
level of u-PAR on the cell surface was not modified by apigenin treatment, as shown by flow cytometry, zymography, and RT-PCR, the decrease in cell-associated u-PA could be directly attributed to the reduced u-PA expression. It has been demonstrated that MAPK pathways were involved in the regulation of u-PA gene expression (35). Thus we observed an inhibition of u-PA expression that is consistent with apigenin being an MAPK signaling pathway inhibitor.

MMP-9 secretion was also affected by apigenin treatment. Under standard culture condition, MDA-MB-231 cells secreted a detectable level of MMP-9, which was upregulated by PMA treatment. Our results show that apigenin, although without effect on the basal secretion of MMP-9, totally abolished the response to PMA stimulation.

Our results are in agreement with the observations that apigenin can block the EGF-induced upregulation of MMP-9 secretion by inhibiting MAPK signaling pathways in breast epithelial tumor cells (27). A recent study demonstrated that the invasiveness and production of MMP-9 were correlated and that they were regulated via protein kinase C, an activator of the MAPK pathway (36). The inhibition of PMA-induced upregulation of MMP-9 may therefore also result from blockade of the MAPK signaling pathway, inasmuch as the tumor promoter PMA activates all three MAPK subfamilies. In a human squamous cell carcinoma cell line, PMA-enhanced MMP-9 secretion and in vitro invasiveness were associated with a strong activation of the p38 MAPK and its downstream target, MAPK-activated protein kinase-2. Inhibition of p38 MAPK by a specific inhibitor resulted in complete reduction of the PMA-induced MMP-9 secretion, but not of u-PA secretion (37). In a recent study, apigenin significantly inhibited EGF-stimulated release of prostaglandin E2, and this inhibition was also p38 dependent (38).

It may be therefore that the inhibition of the PMA-induced MMP-9 secretion by apigenin observed in our study is also mediated by a p38-dependent pathway. Moreover, MMP-9 gene expression was regulated by transcription factors binding the activator protein-1 (AP-1) binding site (the TRE element) (39), and MAPKs have been suggested to be a critical mediator in AP-1-associated changes (40). This is supported by the fact that u-PA and MMP-9 gene expression was dependent on NF-κB (15,16,28), that NF-κB interacts with transcription factors such as AP-1 (41), and that MAPK signaling pathways regulate NF-κB activity (19,20). Although apigenin was shown to inhibit some steps in the signal transduction pathway, an apigenin receptor has not been identified.

Apigenin reduced tumor cell invasiveness through Matrigel, and this seems to correlate with the decrease in protease production. The anti-invasive effects of genistein, an isoconformer of apigenin, was also shown to be mediated through a downregulation of MMP-9 and an upregulation of TIMP-1 (42).

However, the reduced invasiveness and migration of MDA-MB 231 cells induced by apigenin was not simply due to insufficient protease-dependent matrix degradation. Although cell surface saturation by exogenous scu-PA did increase the invasion capacity of the apigenin-treated cells, it was still reduced compared with that of scu-PA-saturated control cells.

In addition, scu-PA saturation could still stimulate cell migration in the absence of matrix regardless of apigenin treatment. It has already been shown that u-PA was able to promote cell motility independently of its capacity to induce matrix proteolysis (43), and u-PA was also shown to be involved in signal transduction pathways leading to modifications of cytoskeleton organization. Apigenin appears therefore to affect MDA-MB231 cell invasion by inhibiting u-PA-related matrix proteolysis and cell motility.

Indeed, apigenin also altered MDA-MB231 adhesive behavior in ECM proteins, and this inhibitory effect on cell adhesion seems not to be related to the u-PA system, inasmuch as it was not influenced by saturating the cells with scu-PA (not shown). Nevertheless, u-PA was found to be implicated in cellular adhesion and migration (44). The reason for this reduced adhesion is unclear, and further study is needed to identify the cell surface adhesive molecules and the matrix proteins involved.

Our results reveal that apigenin, which inhibits the expression of proteases involved in degradation of the ECM, may also regulate other cellular activities associated with the invasive phenotype, including cell migration and cell-matrix adhesion. The decrease in invasiveness may also be due to modification of the ultrastructure of these cells, which was not restored by u-PA. In addition, apigenin treatment resulted in a dose-dependent tumor cell growth inhibition, an effect that was also reported for other cancer cell lines (10,45), and this antiproliferative action was correlated to cell cycle arrest in the G2-M phase (9). However, this effect on cell growth was not specific to apigenin, since other...
flavonoids have also been shown to have antiproliferative effects (46).

In conclusion, our findings indicate that apigenin inhibits tumor cell invasiveness by downregulating protease expression, cell migration, cell adhesion to the ECM, and cell proliferation. This effect is not tumor cell specific, since apigenin also inhibited the growth of endothelial cells and angiogenesis (47,48). Our work supports a direct anticancer effect of apigenin, as a pleiotropic effector, in addition to its effect on angiogenesis. Considering the broad type of apigenin activities in our results, we suggest that interfering with the MAPK signaling pathway and inhibiting the expression of NF-κB-regulated genes represent a promising way to control growth and invasiveness of breast tumors.

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