Apigenin’s anticancer properties and molecular mechanisms of action: Recent advances and future prospectives

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[ABSTRACT] Cancer is a major health concern and leading burden on economy worldwide. An increasing effort is devoted to isolation and development of plant-derived dietary components as effective chemo-preventive products. Phytochemical compounds from natural resources such as fruits and vegetables are responsible for decreasing the risk of certain cancers among the consuming populations. Apigenin, a flavonoid phytochemical found in many kinds of fruits and vegetables, has been shown to exert significant biological effects, such as anti-oxidant, anti-inflammatory and most particularly anti-neoplastic properties. This review is intended to summarize the most recent advances in the anti-proliferative and chemo-preventive effects of apigenin in different cancer models. Analysis of the data from the studied cancer models has revealed that apigenin exerts its anti-proliferative effects through multiple and complex pathways. This guided us to discover some controversial results about the exact role of certain molecular pathways such as autophagy in the anticancer effects of apigenin. Further, there were cumulative positive evidences supporting the involvement of certain pathways such as apoptosis, ROS and DNA damage and repair. Apigenin possesses a high potential to be used as a chemosensitizing agent through the up-regulation of DR5 pathway. According to these preclinical findings we recommend that further robust unbiased studies should consider the possible interactions between different molecular pathways.

[KEY WORDS] Apigenin; Anticancer; Flavonoid; Mechanism of action


Introduction

Cancer, the second leading cause of death, is a major health concern worldwide. The typical treatment measures involve a combination of surgery, radiation, and chemotherapy. Chemotherapeutic agents are mostly cytotoxic drugs that affect cancer and normal fast growing cells equivalently. There has been growing interest in developing naturally occurring phytochemical compounds for cancer therapy [1]. Apigenin (4′, 5, 7-trihydroxyflavone) is a natural compound belonging to the flavone class found in various plants constituting the aglycone of many naturally occurring glycosides like apigetrin, vitexin, and isovitexin (Fig. 1). Many fruits and vegetables are rich in apigenin; among them are parsley, celery, and chamomile tea, in which apigenin constitutes the highest percentage (68%) among the total flavonoids [2]. During the past decades, serious efforts have been made to explore the potential health beneficial effects of apigenin. It has been found to have antioxidant, anti-inflammatory, anti-bacterial, antiviral, antifungal, and anticancer effects [3-11]. Its anticancer properties is noticed to be through its ability to halt cancer growth in a wide range of cancers, by a variety of mechanisms, including liver, pancreatic, colorectal, blood, prostate, breast, lung, thyroid, skin, neck and head, and bone cancers. Several researchers have already reviewed and analyzed the potential effects of apigenin but they were mostly focused on individual diseases. For instance, Venigall et al. have recently summarized its anti-neurodegenerative effects and its role in the treatment of Alzheimer disease [2]. In a similar fashion, the potential anticancer effects of apigenin in the treatment of melanoma are discussed recently, including two reviews about the phytochemicals and flavonoids used to treat melanoma by Harish et al. and Liu-Smith et al. [12-13].
Further the therapeutic potentials of apigenin against breast cancer through careful exploration of data from five in vitro and in vivo studies with the corresponding underlying molecular mechanisms are also assessed [14]. Another evaluation by Bao et al. has specifically discussed apigenin’s anticancer properties and mechanism of action in head and neck cancers [15]. The impact of apigenin on gastrointestinal cancers is another example. Shukla et al. have published detailed analytical reviews on apigenin’s potential anticancer effects in a variety of cancers [17-18]. Throughout the last six years, a considerable number of in vitro and in vivo preclinical studies concerning apigenin’s role as a therapeutic anticancer agent have been performed. However there were no publications to discuss the recently studied effects and their underlying mechanisms of actions. Hence a contemporary and updated analysis becomes essential. The current review is intended to cover the most recent advances in apigenin therapeutic effects against a variety of cancers that have been described during the last six years. To explore the proposed mechanisms of action we compared different pathways reported with different cancer models. This review article would contribute to a better understanding about how apigenin exert its effects, pointing out the missing points needed to be clarified in future preclinical and clinical studies.

Fig. 1 Chemical structure of apigenin

Recent Advances of Apigenin’s Anticancer Effects in Various Tumor Models

In the current review, we tried our best to include all the recent in vitro and in vivo preclinical studies regarding apigenin role in the treatment of cancer. The collection of data was limited to the period between 2010 up to June 2016. Then the reports were subsequently classified according to the cancer types. The main data of each research were described briefly followed by analysis and comparison for key signaling and molecular mechanisms to elucidate the exact key signaling pathways by which apigenin exert its anticancer effects.

Liver cancer

The effects of apigenin on the migration and metastasis in hepatocellular carcinoma (HCC) cell lines in vitro and in vivo that has been recently reported showed its capability of inhibiting the proliferation, migration, and invasion in PLC and Bel-7402 human HCC cells in a dose-dependent manner. Through a pathway involved the reduction of Snail and NF-κB expression, apigenin upturned increases in epithelial-mesenchymal transition (EMT) marker levels, improved cellular adhesion, controlled actin polymerization and cell migration, and inhibited invasion and migration in HCC cells [19]. Yet in other recent study apigenin was showed to exert its cytotoxic effects in HCC through a direct targeting of their mitochondria that was selectively to the cancer cells. This has been confirmed by the elevated mitochondrial parameters such as, mitochondrial membrane potential (MMP), reactive oxygen species (ROS) level, mitochondrial swelling and cytochrome c expulsion, only in cancer cells model [20].

Apigenin’s chemosensitizing effect has also been demonstrated as it can potentiate the cytotoxicity of 5-FU via inhibition of ROS-mediated drug resistance and concurrent activation of the mitochondrial pathways of apoptosis [21]. Apigenin was confirmed to have sensitizing effect in hepG2 cells to TRAIL-induced cell death. The synergistic induction of apoptosis by the combined use of apigenin with TRAIL was confirmed by examining the characteristic morphology changes of apoptosis, PARP-cleavage, and activation of effector caspases. The use of Z-VAD-fmk, a pan-caspase inhibitor, constrained the enhanced cell death by combined treatment of apigenin and TRAIL, indicating that a caspase-dependent pathway is involved in apigenin/TRAIL-mediated apoptosis. Further, there was up-regulation of DR5 cell surface expression and the treatment with DR5 blocking chimera antibody attenuated the synergistic effect of apigenin/TRAIL. ERK activation was involved in the induction of DR5 expression and the Inhibition of ERK1/2 by U0126 considerably reduced the apigenin/TRAIL-induced DR5 expression and apoptosis [22].

Breast cancer

The most common cancer among women in the western world is breast cancer, constituting the second leading cause of cancer-related deaths in women. Breast cancer is one of the cancers with a lower incidence in Asia, compared to Western countries, which has been attributed to the typical Asian food and dietary habits that are rich in flavonoid-containing plants, which is supposed to have anticancer effects [19]. In a very recent study apigenin from Murraya koenigii leaf extract was proved to reduce tumor growth through the inhibition of the endogenous 26S proteasome activity in MDA-MB-231 cells in a dose dependent manner. [23]. It is also tested against MDA-MB-231 cell line at a non-apoptotic inducing concentration and was capable of inhibiting cell proliferation and induction of cell cycle arrest at the G2/M phase. This has been confirmed by Immunoblot analysis that indicated the suppression in the expression of cyclin A, cyclin B, and cyclin-dependent kinase-1 (CDK1) [24]. The effects of apigenin on proliferation and apoptosis in HER2-overexpressing MDA-MB-453 breast cancer cells as evaluated by Seo et al. showed it was mainly through inhibiting STAT3 signaling. This was evident from the up-regulated levels of cleaved caspase-8 and caspase-3, and the provoked cleavage of PARP, induction of extrinsic apoptosis, blocking the phosphorylation of JAK2 and STAT3 in addition to inhibiting CoCl2-induced VEGF secretion and decreased the nuclear staining of STAT3 [25]. Apigenin sup-
pressed the proliferation and clonogenic survival of the human breast cancer T47D and MDA-MB-231 cell lines, in a dose- and time-dependent manner. The death of these cells is due to apoptosis associated with increased levels of Caspase3, PARP cleavage and Bax/Bcl-2 ratios. In addition, the apigenin-treated cells exhibited autophagy, as depicted by the appearance of autophagosomes under fluorescence microscopy and the accumulation of acidic vesicular organelles (AVOs) by flow cytometry. I the same study the combined treatment with the autophagy inhibitor, 3-methyladenine (3-MA), significantly improved apoptosis induced by apigenin, which is accompanied by an increase in the level of PARP cleavage. These results suggest that in addition to the induction of apoptosis, apigenin induces autophagy, which possess cytoprotective role and the use of apigenin as anti-cancer agent can be augmented through the combined use of autophagy inhibitor [26].

**Thyroid cancer**

The treatment of human 8505C and CAL62 ATC (Anaplastic Thyroid Carcinoma) cell lines with apigenin shows synergistic cytotoxic effect with TRAIL through regulation of Becl family proteins, while the suppression of AKT potentiates the cytotoxicity [27]. The anti-neoplastic effects of apigenin on papillary thyroid carcinoma (PTC) BCPAP cells involve autophagy pathway, which is dose-dependent. The mechanism involved the stimulation of reactive oxygen species (ROS) production, and induction of DNA damage, leading to G2/M cell cycle arrest followed by autophagic cell death. The use of 3-MA, an autophagy inhibitor, saved the cells from apigenin-induced cell death [28].

**Colorectal cancer**

In colorectal cancer cells (SW480), apigenin in a dose-dependent manner significantly suppressed cell proliferation, migration and invasion and organoid growth by inhibiting the Wnt/β-catenin signaling pathway [29]. In three kinds of colorectal adenocarcinoma cell lines, SW480, DLD-1 and LS174T, apigenin proved to possess anti-proliferation, anti-invasion and anti-migration properties, with mechanisms involving the up-regulation of TAGLN, down-regulation of MMP-9 expression through decreasing phosphorylation of Akt at Ser473 and in particular Thr308 to prevent cell proliferation and migration [30]. In an attempt to investigate the effects of apigenin on the growth of colorectal carcinoma xenografts in nude mice derived from SW480, it has been found to enhance the expression of FADD and stimulate its phosphorylation, which causes apoptosis of cancer cells, and inhibiting their growth [31]. Apigenin on the other hand has apoptosis- and autophagy-inducing effects in HCT116 colon cancer cells. It was shown that autophagy plays a cytoprotective role in these cells, and the combination of autophagy inhibitor 3-methyladenine (3-MA) significantly enhances the apoptotic effect of apigenin. This study suggested the use of autophagy inhibitor in combination with apigenin to treat colorectal cancers [32]. Turktekin et al. had reported earlier a study on colon cancer model (HT 29), their results confirmed that treatment with apigenin alone would result in cell cycle arrest by activating the caspase cascade and induction of apoptosis. The study also investigated the potential synergistic effect of apigenin with 5-FU, which showed further improved activity [33].

**Prostate cancer**

In human prostate cancer PC-3 and 22Rv1 cells, apigenin was found to directly bind with IKKα, attenuates IKKα kinase activity and suppresses NF-κB/p65 activation much more effectively than IKK inhibitor, PS1145. This led to inhibition of cell proliferation, invasiveness and decrease in tumor growth [34]. The same authors earlier reported that apigenin could be an effective drug for treating prostate cancer in vitro using (PC-3 and DU145 cells) and in vivo xenografts nude mouse model, the underlying mechanism involved targeting inhibitor of apoptosis proteins and Ku70-Bax interaction in the induction of apoptosis [35]. In another study, prostate cancer (PC-3) cells were tested with either apigenin or bortezomib, and the proliferation inhibition was correlated with proteasomal biochemistry, ER-degradation, and cell apoptosis. Apigenin shows more selectivity in inhibiting proteasomal degradation of tumor suppressor ER-β by exclusively inhibiting chymotrypsin-like activity of proteosome, blocking its assembly via PSMA5 and inhibiting USP14 enzyme activity, resulting in cancer cell apoptosis. Unlike bortezomib, apigenin’s actions are associated with subtle, specific, distinctive mechanism capable of abstaining drug resistance [36]. Sharma et al. have shown that apigenin is freely taken up by both normal prostatic epithelial cells and prostate cancer cells, incorporated into their nuclei, allowing its interference with nucleic acid bases that may be responsible for its antioxidant and chemo-preventive activities [37]. Another earlier report by Pandey et al. confirmed that treating PC-3 and 22Rv1 cells with apigenin inhibits class I HDACs, mainly HDAC1 and HDAC3, resulting in reversal of aberrant epigenetic events that promote malignancy [38]. Apigenin sensitizing effect in prostate cancer to the treatment with recombinant human Apo2L/TRAIL was suggested to be through binding and inhibition of ANT2 that up-regulates DR5 and enhancement of apoptosis [39].

**Bladder cancer**

Apigenin has been proven to have potential anticancer activity against T-24 cells in vitro. The results of the study proposed that apigenin inhibits T-24 cells proliferation via blocking cell cycle progression and inducing apoptosis. The flow cytometry and Western blot confirmed that apigenin-mediated subG1 phase accumulation was also associated with a rise in the phospho-p53, p53, p21, and p27 levels, a reduction in the Cyclin A, Cyclin B1, Cyclin E, CDK2, Cdc2, and Cdc25C levels, therefore blocking cell cycle progression. Further, apigenin increased the Bax, Bad, and Bak levels, but decreased the Bel-XL, Bel-2, and Mcl-1 levels, consequently activated the mitochondrial apoptotic pathway (re-
lease of cytochrome c and activation of caspase-9, caspase-3, caspase-7, and PARP). Further analysis demonstrated that apigenin increased the ROS levels and depleted GSH in T-24 cells at 12 h \[40\].

**Pancreatic cancer**

The effect of apigenin on pancreatic cancer was investigated by Wu et al. Apigenin reduced cell growth and induced apoptosis. Apigenin down-regulated the basal and TNF-α-induced NF-κB DNA binding activity, NF-κB transcription activity, inhibitor of NF-κB (IkB) protein degradation. Together with the translocation of p65 and p50, accompanied with the blockade of IkB kinase (IKK)-α/β activity. Further, IKK blockage potentiated the anticancer efficiency of apigenin while IKK-β overexpression reduced apigenin anticancer effects. In addition, the administration of apigenin (30 mg·kg\(^{-1}\)) inhibits cancer growth and IKK-β activation in nude mouse xenografts model of pancreatic cancer. These results suggest the use of apigenin as an effective agent for the treatment of pancreatic cancer \[41\]. Among a group of flavonoids, phenolic acids, ascorbic acid and limonoids that were tested for the possible cytotoxic effects on BxPC-3 and Panc-1 cells, apigenin is the most potent one. It induces pancreatic cell death through inhibition of the glycogen synthase kinase-3β/nuclear factor kappa B signaling pathway. Cell cycle arrested at G2/M phase accompanied by the reduction in the expression of cyclin B1. Apigenin stimulates the mitochondrial pathway of apoptosis and modifies the expression of apoptotic proteins. In addition, it highly up-regulated the expression of cytokine genes IL17F, LTA, IL17C, IL17A, and IFNβ1 in BxPC-3 cells, potentially contributed to the noticed anticancer properties \[42\].

**Oral cancer**

The anti-proliferative effects of apigenin have been shown in a tongue cancer-derived cell line (SCC-25) and a keratinocyte cell line (HaCaT), inhibiting cell growth in both cell lines particularly in SCC-25. Further, apigenin induces apoptosis and arrests cell cycle at both G0/G1 and G2/M phases in SCC-25 cells. Reduction in the expression of cyclins D1 and E and inactivation of CDK1 have been observed upon apigenin treatment as well, suggesting that apigenin might be a promising chemopreventive agent due to its cytotoxic actions and its ability to act as a cell cycle modulating agent at multiple levels \[43\].

**Lung cancer**

The assessment of apigenin effects on lung carcinoma cell line A549 has been focused on apoptosis as its supposed molecular mechanism of action, revealing its ability to induce apoptosis that involved mitochondrial pathway associated with apigenin-DNA interaction, DNA fragmentation, ROS accumulation, cytochrome c release and mitochondrial transmembrane potential depolarization, up-regulation of Bax, caspase 3, 9, and PARP, down-regulation of Bcl-2 and cytochrome c in the mitochondrial fraction. Apigenin indirectly targeted mitochondrial oxidative phosphorylation system as evident from the results of mitochondrial inner membrane swelling measurements, intracellular ADP/ATP ratio, and ATPase activity. Its action might be through an upstream step to activate the mitochondrial apoptotic pathway \[44\]. In another study using the same cell line A549, apigenin induces apoptosis and cell death and the target identification investigations show that it depolymerizes microtubules and inhibits reassembly of cold depolymerized microtubules of A549 cells. Further, the concomitant use of apigenin with curcumin, another natural anti-tubulin agent, shows synergistic activity. They synergistically induce cell death and apoptosis and also block cell cycle progression at G2/M phases in A549 cells \[45\].

**Skin cancer**

The effects of apigenin in the treatment of human malignant melanoma cell line A375 have been investigated by Das et al. They have demonstrated that these cells were highly sensitive to apigenin-induced apoptosis. The apoptotic process involves mitochondrial pathway associated with apigenin-DNA interaction, DNA fragmentation, cytochrome c release and mitochondrial transmembrane potential depolarization, Bax, caspase 3, 9, and PARP up-regulation, down-regulation of Bcl-2 as well as cytochrome c in the mitochondrial fraction. Apigenin directly targets and impairs mitochondrial function in A375 cells, through the interference with their oxidative phosphorylation system \[44\].

**Bone cancers**

Human osteosarcoma U-2 OS cells have been used by Lin et al. to study the anticancer effects of apigenin on bone cancers. Their study has clarified that apigenin significantly decreases cell viability and provokes apoptosis through the activations of caspases-3, -8, -9, and BAX and enhancing the release of AIF in U-2 OS cells as well as inhibiting tumor growth in in vivo xenografts tumors models (nude mice bearing U-2 OS) \[46\].

**Blood cancers**

Gonzalez-Mejia et al. have investigated the effect of apigenin on the regulation of Hsp27 (small heat-shock protein) in leukemia cells. High expression of Hsp27 in leukemia contributes to the resistance of these cells to cancer treatments. Their study showed that apigenin does not affect the expression of Hsp-27 but induces a bimodal phosphorylation on Ser78 and Ser82. In addition, apigenin-induced PKCδ activity found to be mediated by p38. The phosphorylation of Hsp27 drastically improved the susceptibility of leukemia cells to apigenin-induced apoptosis. The study identified a complex signaling network regulating the cytotoxic effect of apigenin through Hsp27 phosphorylation \[47\]. Yet another study analyzed the molecular effects of apigenin treatment in two types of leukemia, the myeloid and erythroid subtypes. Apigenin prevented proliferation in both cell lines through cell-cycle arrest in G2/M phase for myeloid HL60 and G0/G1 phase for erythroid TF1 cells. The JAK/STAT pathway was one of major targets of apigenin in both cell lines. Apigenin constrained PI3K/PKB pathway in HL60 and provoked
caspase-dependent apoptosis. On the other hand, no apoptosis was detected in TF1 cells, but commencement of autophagy was observed. These effects had lowered the susceptibility of cancer cells toward vincristine treatment. Hence, this study suggested apigenin could act as cytoprotective agent through the induction of autophagy that should be considered during cancer treatment \[48\]. In an earlier study Zhao et al. reported a study about apigenin effects in multiple myeloma (MM) and primary multiple myeloma cell lines model. The study confirmed that apigenin revealed cytotoxicity against both cell lines, the mechanism of which revealed that the therapy had the potential of inducing apoptosis in MM. The mechanism of apoptosis was suggested to be through the up-regulation of both TNF-R and TRAIL-R signaling pathways \[50\].

**The Mechanisms of Action for Apigenin Cancer Therapy**

In order to summarize the mechanisms of action and molecular pathways involved in various cancer models, Table 1 is constructed to include all the relevant information for the related cell lines or cancer models. The variability among the pathways is obvious. Amongst the common mechanisms and key signaling pathways is the involvement of apoptotic pathway demonstrated by the increase in the apoptotic proteins. In addition, the role of ROS involvement has been demonstrated in many cancer models including liver, thyroid, Bladder, lung, and skin cancers. Autophagy induction on the other hand is evidenced in breast, leukemia, colon, and thyroid cancers. The interference with nuclear proteins is one of the pathways in several cancer models such as thyroid, pancreatic, skin, lung and prostate cancers. However most of the other key signaling and pathways are more specific for cancer models under investigation (Table 1).

**Table 1  Molecular mechanism by which apigenin interacts with various types of tumor**

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Key signaling</th>
<th>Cell line</th>
<th>Year</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Liver cancer</td>
<td>↓Snail and NF-κB ↑EMT, ↓proliferation, migration, and invasion Selective direct targeting of mitochondria. ↑MMP and ROS Chemosensitizing agent with 5FU ↓ROS-mediated drug resistance Chemosensitizing agent with TRAIL ↑PARP-cleavage, ↑ effector caspases ↓DR5 expression through ↓ERK</td>
<td>PLC, Bel-7402</td>
<td>2016</td>
<td>10</td>
</tr>
<tr>
<td>Thyroid cancer</td>
<td>Chemosensitization with TRAIL by regulation of Bcl2 family proteins Induction of autophagy by ↑ROS production, inductions of DNA damage.</td>
<td>Human 8505C &amp; CAL62 ATC</td>
<td>2015</td>
<td>27</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>↓proliferation, migration and invasion by ↓Wnt/β-catenin signaling pathway ↓proliferation, invasion &amp; migration by ↑TAGLN, ↑MMP-9 ↑PADD expression and stimulates its phosphorylation and induce apoptosis</td>
<td>SW480 cells</td>
<td>2016</td>
<td>29</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>↓IKKα kinase activity, ↓NF-κB/p65 ↓cell proliferation, ↓ invasiveness Induction of apoptosis by targeting inhibitor of apoptosis proteins and Ku70-Bax interaction</td>
<td>PC-3, 22Rv1</td>
<td>2015</td>
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The antiproliferative role of apigenin; most of them lead to agents. Multiple pathways have been shown to be involved in reversal of aberrant epigenetic events that promote malignancy. Chemosensitizing effect to TRAIL through binding and of ANT2 that DR5 and enhancement of Apo2L/TRAIL-induced apoptosis. Nevertheless, it is critically essential to confirm the subsequent theory with experimental data in cell cultures and applicable animal models prior to initiation of clinical trials. From the recently reported activity of apigenin in prostate cancer cells PC3 (Table 1), we have realized that it is mostly through multiple pathways such as the binding with IKKα, which suppresses NF-xB/p65 activation, and targeting inhibitor of apoptosis proteins and Ku70-Bax interaction in the induction of apoptosis. Both results have been recorded with PC3 cell line and shown that the effect is selective in inhibiting proteasomal degradation of tumor suppressor ER-β, resulting in cancer cell apoptosis. Furthermore, Sharma et al. have utilized PC3 cell line as well and shown that apigenin interferes with nucleic acid bases that may be

### Discussion and Conclusions

The most logical approach in drug development is to test it on specific molecular and cellular targets in a suitable animal model to determine the efficacy ahead to the initiation of clinical trials. Most of such information can be gained from epidemiological studies, which can provide esteemed recommendations for the development of chemo-preventive agents. Nevertheless, it is critically essential to confirm the subsequent theory with experimental data in cell cultures and applicable animal models prior to initiation of clinical trials. Apigenin is a common plant-derived flavonoid abundantly obtainable from fruits and vegetables and has been shown to have remarkable and promising effects as a potent anticancer agent. It is an active molecule against many kinds of cancer used alone or in combination with other chemotherapeutic agents. Multiple pathways have been shown to be involved in the antiproliferative role of apigenin; most of them lead to induction of apoptosis and/or autophagy. Comparing apigenin chemopreventive effects and pathways in certain cancer models can bring a better idea about the most probable mechanism by which these actions are initiated. This can be of particular importance when these studies performed in different institutions to eliminate the possibility of bias. From the recently reported activity of apigenin in prostate cancer cells PC3 (Table 1), we have realized that it is mostly through multiple pathways such as the binding with IKKα, which suppresses NF-xB/p65 activation, and targeting inhibitor of apoptosis proteins and Ku70-Bax interaction in the induction of apoptosis. Both results have been recorded with PC3 cell line and performed by the same group of researchers. Further, Singh et al. used the same cell line and shown that the effect is selective in inhibiting proteasomal degradation of tumor suppressor ER-β, resulting in cancer cell apoptosis. Furthermore, Sharma et al. have utilized PC3 cell line as well and shown that apigenin interferes with nucleic acid bases that may be
responsible for its antioxidant and chemo-preventive activities. Moreover, Pandey et al. have confirmed its inhibition to class I HDACs, resulting in reversal of aberrant epigenetic events that promote malignancy in the same PC3 cell line. Another example to compare different pathways in the same cell line is the effects of apigenin in colorectal cell line SW480 (Table 1); this model similarly showed three different pathways could be involved in the antiproliferative effects of apigenin, as the first study showed to be through the inhibition of Wnt/β-catenin signaling pathway, while the second study proved to be through the up-regulation of TAGLN and the down-regulation of MMP-9 and the third study reported to be through the activation of FADD expression and stimulation of its phosphorylation and induction of apoptosis.

From these findings it is obvious that different pathways are confirmed to be involved in the noticed effect even in cases where the same group of researchers performed the study. It is also clear that in each study the focus was from the beginning on the involvement of the pathway of interest in the resulted effect and the study has either to approve the hypothesis or deny it. Another important opinion is that even when some pathway is confirmed to be involved there is always high possibility of not being the only one responsible for the designated effect, or even if apigenin directly induced it. This brings hypotheses that apigenin may initiate a cascade of events that involve one or more of these pathways. This hypothesis was confirmed when lung cancer A549 cells and skin cancer A375 cells were treated with apigenin as the study proved that the apoptotic effect in A549 cells was not directly induced by apigenin but rather by an upstream step that may lead to activate the mitochondrial apoptotic pathway. However, in A375 cell line the study proved apigenin could directly target and impair the mitochondrial function by breaking down their oxidative phosphorylation system.

Similarly comparing the role of certain pathway or mechanism of action in different cancer models on the other hand would be worthwhile to fully understand this pathway and may lead us to focus on treating certain kind of cancers accordingly where such pathways are expected to play a distinctive role in cancer prognosis. The induction of autophagy in apigenin treatment is one of the pathways with distinctive role behind its anticancer potential. It induces autophagy in breast, leukemia, colon, and thyroid cancers, and leukemia. Autophagy has cytoprotective function against breast, leukemia and colon cancer cells, suggesting the use of autophagy inhibitor as an effective means to improve the apoptotic effect induced by apigenin. However, the role of autophagy in thyroid cancer seems to be the opposite as the use of the autophagy inhibitor rescued the cells from apigenin-induced cell death. These controversial results make another challenge to understand the exact mechanism of cell death induction that apigenin imposes on the studied cell lines.

Additionally, there were cumulative data from hepatic and prostate cancers indicate that apigenin sensitizing effect can be through the up-regulation of DR5 pathway. This would contribute to enhance the use of apigenin as an adjuvant chemotherapeutic agent, particularly in the treatment of drug resistant cancers.

**Future Perspectives**

After analyzing the most recent advances in apigenin anticancer potentials some controversial results and missing point are highlighted. These should have the priority in the future proposed researches in introduction of this drug to the clinic. They include primarily the involvement of multiple different pathways in the anticancer effect of apigenin within the same cell lines that lead us to conclude it is wise to have more extensive unbiased studies to explore and evaluate all these findings as well as the possibility of interference among these pathways before commencing any clinical studies. Further, the exact role of certain pathway should be well known before commencing any clinical study accordingly; whether autophagy induction in apigenin treated cancer models contributes to the cell death or cell survival is still to be elucidated in future studies. Additionally, although there are some evidences supporting its use as a chemosensitizing agent, a better understanding of the mechanism of action is of great importance in clinical use as adjuvant therapy with other chemotherapeutic agents. These findings should be well considered in the future studies exploring apigenin anticancer potentials to fully elucidate them and to accelerate its introduction to clinics.

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