

Molecular targets of luteolin in cancer

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Many food-derived phytochemical compounds and their derivatives represent a cornucopia of new anticancer compounds. Despite extensive study of luteolin, the literature has no information on the exact mechanisms or molecular targets through which it deters cancer progression. This review discusses existing data on luteolin's anticancer activities and then offers possible explanations for and molecular targets of its cancer-preventive action. Luteolin prevents tumor development largely by inactivating several signals and transcription pathways essential for cancer cells. This review also offers insights into the molecular mechanisms and targets through which luteolin either prevents cancer or mediates cancer cell death. *European Journal of Cancer Prevention*

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Introduction

Compounds of natural origin could lead to new, innovative therapeutic agents for cancer. Several promising new anticancer agents have been developed and used in the clinic on the basis of their selective molecular targets (Rengarajan *et al.*, 2014). Yet, the progress of modern technology enables us to design and synthesize drug molecules for specific molecular targets. Therefore, we can shift our attention from chemically synthetic drugs to purely natural ones (Ortholand and Ganesan, 2004; Montaser and Luesch, 2011). Luteolin (3,4,5,7-tetrahydroxy flavone) is a natural flavonoid present in several plants. Vegetables and fruits rich in luteolin include carrots, broccoli, onion leaves, parsley, celery, sweet bell peppers, and chrysanthemum flowers (Miean and Mohamed, 2001; Sun *et al.*, 2007; Chen *et al.*, 2012b; Lim *et al.*, 2013). Like other flavonoids, luteolin is mainly glycosylated in plants. During digestion and intestinal absorption, luteolin's glycosylated form is mainly hydrolyzed to free luteolin (Hempel *et al.*, 1999). However, during passage through the intestinal stroma, some luteolin can reconvert into its glycosylated form (Shimoi *et al.*, 1998). Luteolin is a heat-stable reagent that degrades relatively little during cooking (Le Marchand, 2002). Luteolin has potent activity against cancer, inflammation, and oxidation, and it can reverse multidrug resistance (MDR) in many types of cancer cells (Park *et al.*, 2012; Ou *et al.*, 2013; Chen *et al.*, 2014; Jeon *et al.*, 2014; Khan *et al.*, 2014). Alone or with other chemotherapeutics, luteolin can sensitize MDR cancer cells (Dellafiora *et al.*, 2014). It can also ameliorate the cytotoxicity that various chemotherapy drugs can cause. Despite luteolin's well-documented anticancer properties, exactly how these work remains unclear. To the best

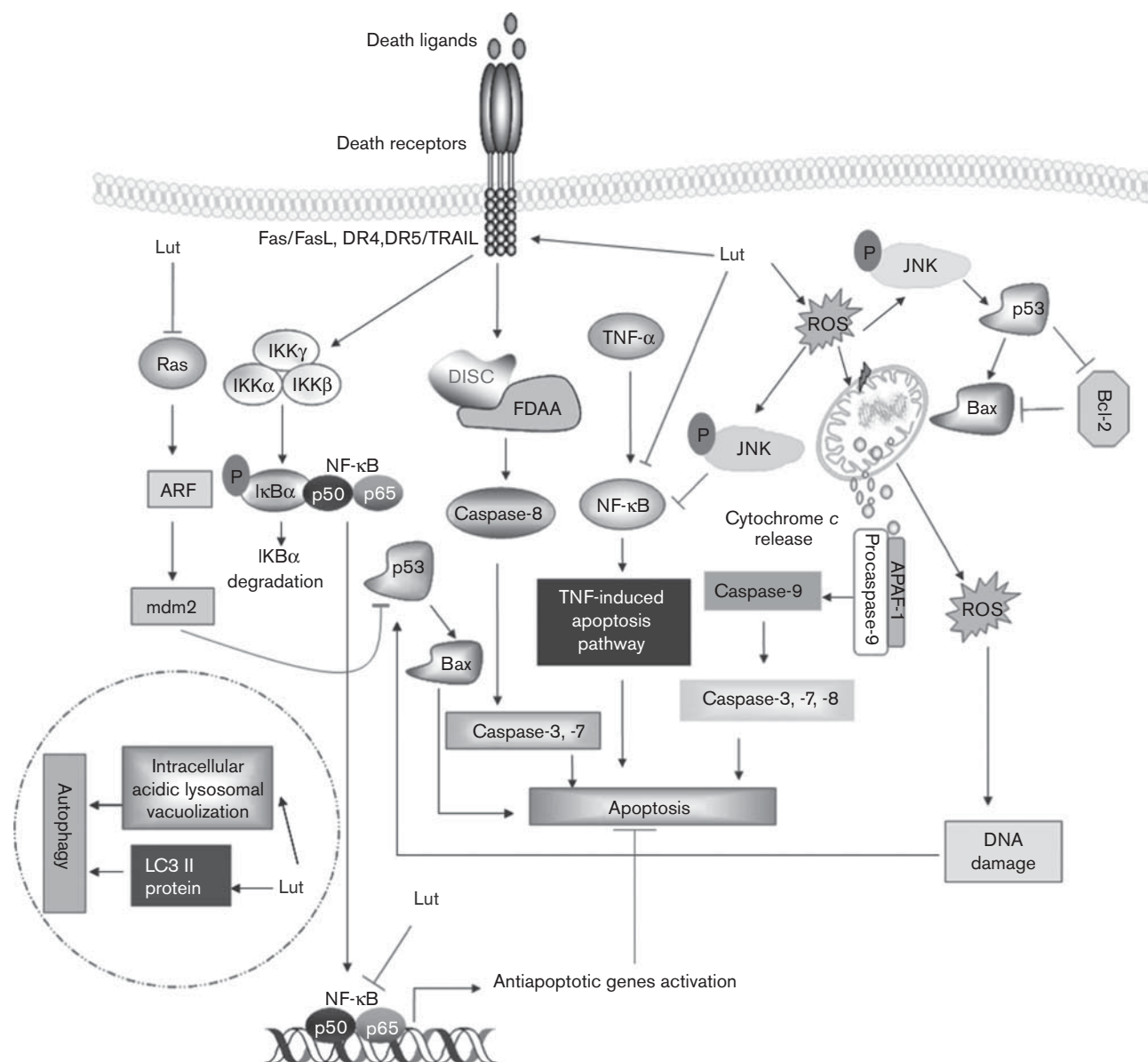
of my knowledge, no seminal review has determined the potential mechanisms of luteolin's anticancer activities, except that published by Lin *et al.* (2008).

Apoptosis pathways

Apoptosis occurs through two major pathways: intrinsic and extrinsic. The intrinsic apoptosis pathway operates by modulating mitochondrial membrane potential, which releases cytochrome *c* and inhibits the expression of antiapoptotic proteins Bcl-2 and Bcl-xL. The extrinsic apoptosis pathway operates through activation of caspase-3, -7, -8, and -9 and enhanced expression of death receptors and their downstream factors, such as DR4, DR5, tumor necrosis factor receptor apoptosis-inducing ligand (TRAIL), and Fas/FasL (Ham *et al.*, 2014). When the signal of apoptosis is received, Fas-associated death domain binds and recruits the death-induced signaling complex, forming initiator caspases-8 and -10 (Park *et al.*, 2013b). Any alteration or interruption in the mitochondrial membrane could activate both intrinsic and extrinsic apoptosis pathways by triggering caspase activities; promoting imbalance of the Bax/Bcl-xL ratio; and decreasing the expression of p21, survivin, Mcl-1, and mdm2 proteins (Chang *et al.*, 2005; Lim do *et al.*, 2007; Chen *et al.*, 2012a). Researchers have implicated the endoplasmic reticulum as a third subcellular compartment involved in apoptosis (Nakagawa *et al.*, 2000; Rao *et al.*, 2004).

In many ways, luteolin can trigger both intrinsic and extrinsic apoptosis pathways in a variety of human cancer cells (Fig. 1). In part, luteolin can arrest the cell cycle and then induce apoptosis. For instance, in the SH-SY5Y neuroblastoma tumor cell line, luteolin arrests G₀/G₁ cell cycle growth, accompanied by loss of mitochondrial

Fig. 1



Mechanisms of luteolin (Lut)-induced apoptosis and autophagy in cancer cells. Luteolin mediates both the intrinsic and the extrinsic apoptosis pathways. Luteolin triggers the intrinsic apoptosis pathway by modulating mitochondrial membrane potential, releasing cytochrome c, and inhibiting the expression of Bcl-2 and Bcl-xL. Luteolin mediates extrinsic apoptosis by activating caspase activities; enhances expression of death receptors and their downstream factors such as Fas/FasL, DR4, DR5, and TRAIL; and suppresses other death receptor survival pathways. Luteolin also inhibits mdm2 activated by Ras; mdm2 expression triggers p53 degradation. p53, a tumor suppressor protein, mediates apoptosis by enhancing Bax levels and reducing levels of antiapoptotic protein Bcl-2. Luteolin can directly mediate apoptosis by mediating DNA damage induced by ROS. DNA damage signaling, in turn, enhances p53 production and activity. Luteolin activates JNK, which inhibits TNF- α -mediated NF- κ B (p65) translocation, promoting TNF- α -induced apoptosis in cancer cells. However, luteolin can mediate autophagy as a cell death mechanism by triggering the intracellular acidic lysosomal vacuolization and accumulation of microtubule-associated LC3 II protein, which in turn enhances autophagy flux. IKK, I- κ B kinase; LC3, light chain-3; NF- κ B, nuclear factor- κ B; ROS, reactive oxygen species; TNF- α , tumor necrosis factor α ; TRAIL, tumor necrosis factor receptor apoptosis-inducing ligand.

membrane potential and apoptosis (Wang *et al.*, 2014). Furthermore, luteolin inhibits SMMC-7721 and BEL-7402 cell proliferation by arresting the cell cycle at the G₁/S phase, enhancing the level of Bax and reducing the level of antiapoptotic protein Bcl-2, leading to apoptosis (Ding *et al.*, 2014). Luteolin can also directly

induce apoptosis by activating JNK, which inhibits the translocation of tumor necrosis factor α (TNF- α)-mediating nuclear factor- κ B (NF- κ B) p65 to the nucleus (Cai *et al.*, 2011). Furthermore, in human non-small-cell lung cancer A549 cells, apoptosis occurs by phosphorylating JNK and inhibiting NF- κ B translocation as a

transcription factor from the nucleus (Hu *et al.*, 2012). Surprisingly, although luteolin increased Bax and caspase-3 expression and upregulated Bcl-2 expression in liver carcinoma cells, it exerted almost no effect on normal liver HL-7702 cells (Ding *et al.*, 2014).

Autophagy

Autophagy is a process of cellular self-eating activated by lysosomal activity caused by nutrient depletion. In addition to its role in maintaining cellular balance under normal physiological conditions, it is also implicated in the development of genetic diseases and drug resistance in cancer cells (Uekita *et al.*, 2013; Gewirtz, 2014; Wang and Wu, 2014). Luteolin-induced autophagy functions as a cell death mechanism (Fig. 1) by accumulating microtubule-associated protein light chain-3 II protein, which in turn enhances autophagy flux (Park *et al.*, 2013a). In metastatic MET4 cells, luteolin stimulated autophagy by triggering intracellular acidic lysosomal vacuolization (Verschooten *et al.*, 2012).

Cell cycle regulation

The cell cycle, arranged in the following phases, leads to cell growth and division:

- (1) In the G₁ phase, the cell grows and chromosomes prepare for replication.
- (2) In the S phase, DNA replicates and chromosomes duplicate.
- (3) The G₂ phase represents the gap between DNA synthesis and mitosis.
- (4) In the M phase (mitosis), nuclear and cytoplasmic division occurs, yielding two daughter cells.

Luteolin can keep several human cancers from growing, but the precise molecular mechanisms are unclear. Figure 2 shows the molecular mechanisms underlying luteolin's antiproliferative activities. Luteolin induces cell cycle arrest and apoptosis by decreasing the expression of AKT, PLK1, cyclin B1, cyclin A, CDC2, CDK2, Bcl-2, and Bcl-xL as well as increasing the expression of Bax, caspase-3, and p21 (Lee *et al.*, 2012; Pandurangan *et al.*, 2013). Luteolin also arrested colon cancer cell growth through Wnt/ β -catenin/glycogen synthase kinase-3 β (GSK-3 β) signaling (Pandurangan *et al.*, 2013). However, luteolin can obviously arrest the cell cycle by suppressing Akt phosphorylation, which dephosphorylates and activates GSK-3 β . Activating GSK-3 β enhances phosphorylation of cyclin D1 at Thr-286, followed by proteasomal degradation (Ong *et al.*, 2010).

Potential molecular targets of luteolin-mediated cell cycle arrest

Insulin-like growth factor 1 (IGF-1) is crucial in cellular growth, proliferation, and apoptosis (Katic and Kahn, 2005; Pollak, 2008). Altered IGF-1 function is implicated

in tumorigenesis, metastasis, and resistance of human cancer cells (Lin *et al.*, 2014). IGF-1 signaling begins when IGF-1 binds with its cell surface receptor, IGF-1R, forming a homodimer signaling complex, phosphorylating IGF-1R, which then phosphorylates intracellular insulin receptor substrate 1 (IRS-1) for its downstream targets (Chitnis *et al.*, 2008; Aleksic *et al.*, 2010). In HT-29 cells treated with luteolin, reduced IGF-1R signaling downregulated the PI3K/Akt and ERK1/2 pathways (Lim do *et al.*, 2012). However, luteolin's inhibitory action on IGF-1 extends beyond inhibiting IGF-1R; it can also inhibit Akt signaling (Fang *et al.*, 2007). Inhibition of Akt signaling in turn dephosphorylates its downstream targets, including p70S6K1, GSK-3 β , and FKHR/FKHRL1 (forkhead human transcription factor like 1). Moreover, in estrogen receptor (ER)-positive tumors and cell lines, IGF signaling can also cooperate with the ER to promote tumor growth and progression, while hindering the efforts of endocrine therapy (Zhang *et al.*, 2011; Mancini *et al.*, 2014). Targeting ER α is a possible mechanism of luteolin's antiproliferative effect (Wang *et al.*, 2012a). Using an ER α -specific small interfering RNA to knock down ER α in MCF-7 cells reduced luteolin's ability to inhibit the growth of MCF-7 cells. This finding suggests that luteolin's inhibitory effect on cancer cell growth may inhibit the IGF-1-mediated PI3K/Akt pathway depending on ER α expression. Thus, the downregulation of the PI3K/Akt and mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) pathways through luteolin's reduction of IGF-1R/ER α signaling pathways may offer promising routes for cancer therapeutic agents.

Fms-like tyrosine kinase 3 (FLT3) is another potential means by which luteolin arrests the cell cycle. In one study, FLT3 was highly overexpressed in most patients with acute myeloid leukemia (Chin *et al.*, 2013). Luteolin suppressed cell proliferation in MV4-11 cells with constitutively activated FLT3, suggesting that luteolin may be a potent FLT3 enzyme inhibitor.

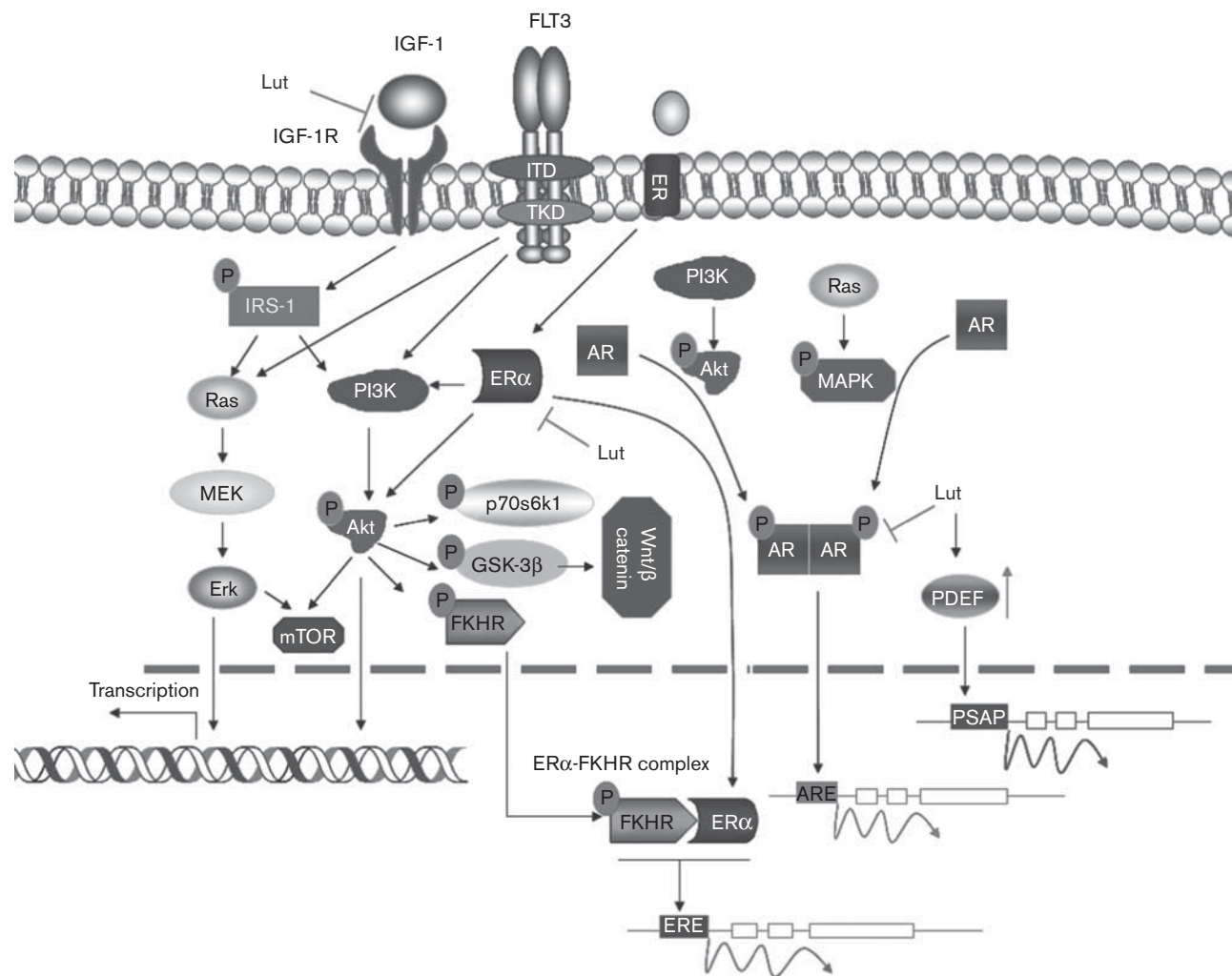
Downregulated androgen receptor expression could be a main mechanism through which luteolin mediates its antiproliferative and anti-invasive effects in LNCaP human prostate cancer cells (Chiu and Lin, 2008). By contrast, luteolin upregulates the expression of prostate-derived Ets factor (PDEF) in LNCaP cells, which acts as an androgen-independent transcriptional activator of the prostate-specific antigen promoter (Tsui *et al.*, 2012).

Molecular targets of luteolin-induced apoptosis

Nuclear factor- κ B-induced and tumor necrosis factor α -induced apoptosis pathway

NF- κ B is synthesized in the cytoplasm and complexed with its inhibitor I- κ B; thus, NF- κ B is released as an inactive form. To activate, I- κ B must undergo phosphorylation, followed by proteasomal degradation of the

Fig. 2

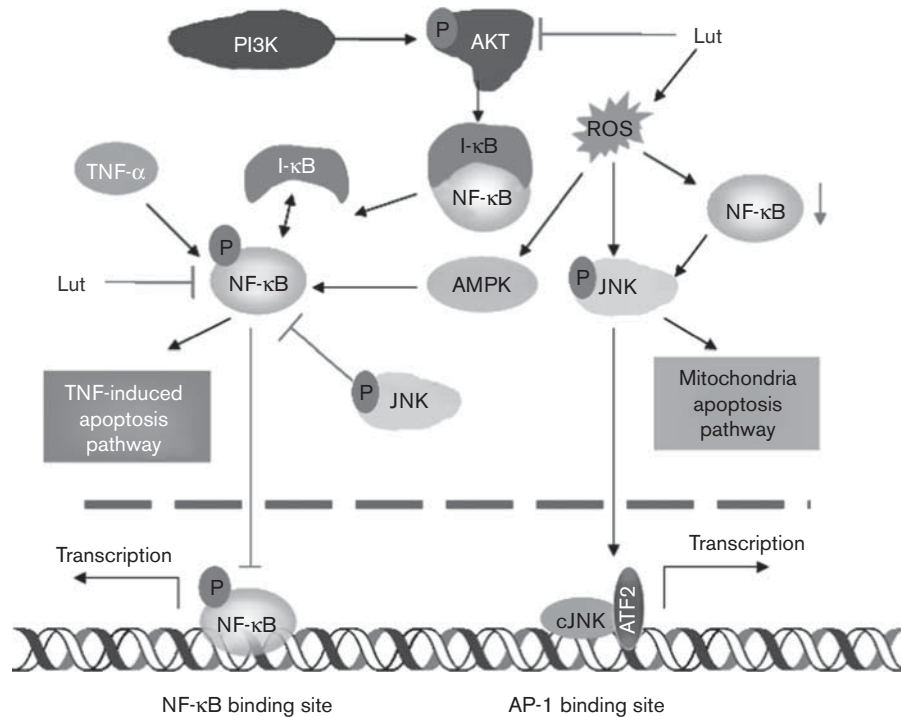


Luteolin (Lut) modulates cancer cell cycle progression. Luteolin's antiproliferative activity is attributed to its ability to inhibit IGF-1 activation, thus preventing the phosphorylation of the intracellular IRS-1 and its downstream targets. Furthermore, luteolin inhibits IGF-1-mediated PI3K/Akt activation by reducing the expression of ER α . Estradiol receptor triggers the PI3K/AKT pathway, mediating FKHR phosphorylation, which functionally associates with ER α and forms a FKHR-ER α complex. Inhibiting AKT activity reduces phosphorylation of its downstream targets, including p70S6K1, GSK-3 β , and FKHR. Luteolin suppresses prostate cancer cell proliferation by downregulating AR expression. Phosphorylation of the cytoplasmic AR by MAPK and AKT enables AR to form dimers and enhances ARE. By contrast, luteolin upregulates the expression of PDEF, which acts as an androgen-independent transcriptional activator of the prostate-specific antigen promoter. AR, androgen receptor; ARE, androgen response element; ER, estrogen receptor; Erk, extracellular signal-regulated kinase; FKHR, forkhead transcription factor; FLT3, Fms-like tyrosine kinase 3; GSK-3 β , glycogen synthase kinase-3 β ; IGF-1, insulin-like growth factor 1; IRS-1, insulin receptor substrate 1; ITD, internal tandem duplications; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; PDEF, prostate-derived Ets factor; PSAP, prosaposin; TDK, tyrosine kinase domain.

NF- κ B-p- κ B complex. The free p-NF- κ B then translocates to the nucleus to transcribe and activate genes to synthesize pro-growth and anti-apoptosis proteins (Lun *et al.*, 2005). NF- κ B is a heterodimer composed of two subunits: the DNA-binding subunit p50 and the trans-activator p65. Phosphorylation of I κ B α is mediated by the I- κ B kinase (IKK) complex, which consists of NF- κ B essential modulators IKK γ , IKK α , and IKK β , degrading I κ B α through a ubiquitin/proteasomal process (Thomas *et al.*, 2009). Degrading I κ B α allows insertion of NF- κ B's two subunits into the nucleus to transcribe and activate target genes.

The NF- κ B transcription factor plays a major role in the development and progression of various cancers (Erez *et al.*, 2013; Wu *et al.*, 2013; Kagoya *et al.*, 2014). In many cancers, TNF- α is one of the most important activators for NF- κ B and plays a paramount role in activating pathways for both cancer cell death and survival. On the one hand, TNF- α 's activation of NF- κ B abolishes TNF-induced cancer cell apoptosis, which plays a marginal role in the development of resistance in cancer cells. On the other, blocking NF- κ B enhances TNF- α 's anticancer activity (Ju *et al.*, 2007). Luteolin can suppress NF- κ B, thus activating TNF- α -induced apoptosis (Fig. 3).

Fig. 3



Mechanism of luteolin (Lut)-triggered TNF- α -induced cancer cell apoptosis. Free p-NF- κ B translocates to the nucleus to mediate the transcriptional activation of genes. Luteolin can suppress the activity of NF- κ B translocation, activating the TNF- α -induced apoptosis pathway. The generation of ROS caused by treatment with luteolin plays a marginal role in suppressing NF- κ B, further enforcing JNK activation. ROS activate the AMPK signaling pathway, which interacts with the NF- κ B pathway, thereby inhibiting NF- κ B DNA-binding activity. Activating JNK activates the mitochondrial apoptosis pathway. Furthermore, luteolin's inhibition of NF- κ B activity augments and prolongs cJNK activation induced by TNF- α . AMPK, AMP-activated protein kinase; AP-1, activating protein 1; ATF2, activating transcription factor 2; JNK/cJNK, c-Jun N-terminal kinase; NF- κ B, nuclear factor- κ B; ROS, reactive oxygen species; TNF- α , tumor necrosis factor α .

A possible mechanism for this process is through its ability to mediate the release of reactive oxygen species, which suppresses NF- κ B and activates JNK, stimulating cancer cells to undergo TNF- α -induced apoptosis (Ju *et al.*, 2007). Hwang *et al.* (2011) suggested AMPK as a novel regulator of NF- κ B in luteolin-induced cancer cell death (Hwang *et al.*, 2011), as inhibiting AMPK activity restored luteolin-inhibited NF- κ B DNA-binding activity.

Reactive oxygen species generation caused by luteolin treatment is the major mechanism through which luteolin activates AMPK (Hwang *et al.*, 2011). However, luteolin can obviously induce apoptosis in human non-small-cell lung cancer A549 cells by phosphorylating JNK, activating the mitochondrial pathways of apoptosis while inhibiting NF- κ B translocation (Hu *et al.*, 2012). Furthermore, luteolin's inhibition of NF- κ B augmented and prolonged TNF- α -induced cJNK activation (Shi *et al.*, 2004). Taken together, these findings indicate that luteolin's sensitization of TNF- α -induced cancer cell death may encompass many cancer types. Interestingly, inhibiting NF- κ B's transcription activity also downregulated the expression of vascular endothelial growth factor (VEGF) mRNA, inhibiting VEGF secretion in pancreatic carcinoma cells

(Cai *et al.*, 2012). This finding suggested that luteolin had potent antiangiogenesis activity.

Tumor necrosis factor receptor apoptosis-inducing ligand

TRAIL is an endogenous protein belonging to the TNF family. TRAIL induces apoptosis in a wide variety of transformed and cancer cells, but has little or no effect on normal cells (Rushworth and Micheau, 2009). Luteolin can sensitize TRAIL-induced apoptosis in both TRAIL-sensitive cancer cells, including HeLa (Horinaka *et al.*, 2005; Shi *et al.*, 2005; Yan *et al.*, 2012) and human 786-O renal cell carcinoma (Ou *et al.*, 2013), and TRAIL-resistant cancer cells (CNE1, HT-29, and HepG2) (Shi *et al.*, 2005). Luteolin is also a potential sensitizer of TRAIL in anticancer therapy against human renal cell carcinoma involving Akt and STAT3 inactivation (Ou *et al.*, 2014). However, the Janus tyrosine kinases (Jak1) and tyrosine kinase 2 (Tyk2) mediate most, if not all, cellular responses to peptide hormones, cytokines, and interferons (IFNs) and are often hyperactivated in tumors (Muller *et al.*, 2014). In fact, neither Jak1 nor Tyk2 has serine activities (Carbone and Fuchs, 2014); thus, they must undergo phosphorylation before they can act.

Luteolin can sensitize the antiproliferative effect of IFN by enhancing phosphorylation of Jak1 and Tyk2, thus ensuring the activation of STAT1/2, which promotes STAT1 accumulation in the nucleus and endogenous IFN- α -regulated gene expression (Tai *et al.*, 2014). Treatment with TRAIL and luteolin markedly reduced the growth of xenograft tumors in animals (Yan *et al.*, 2012). Therefore, luteolin's potent activity to sensitize both TRAIL-sensitive and TRAIL-resistant cancer cells may represent another dimension for the development of new techniques enabling us to conjugate luteolin or use it as a juvenile agent with other anticancer drugs.

Modulation of Wnt/ β -catenin signaling

Wnt/ β -catenin signaling regulates the proliferation and differentiation of many normal and malignant cells (Abdel-Magid, 2014; Draganova *et al.*, 2015; Zhao and Carrasco, 2014). Luteolin's antiproliferative effect on cancer may be attributed to its inhibitory effect on Wnt/ β -catenin signaling. For instance, luteolin decreases the expression of Wnt/ β -catenin/GSK-3 β signaling, arresting the growth of colon cancer cells (Pandurangan *et al.*, 2013). Wnt/ β -catenin/GSK-3 β signaling is also involved in luteolin-prevented azoxymethane-induced cellular proliferation (Pandurangan *et al.*, 2014).

Topoisomerases

Topoisomerases, especially DNA topoisomerases, are among the most desired targets for chemotherapy drugs. Topoisomerase inhibition might correlate with the antioxidant capacity of the flavonoids (Topcu *et al.*, 2008). Chowdhury *et al.* (2002) published the first report on luteolin functionally inhibiting the catalytic activity of topoisomerase. The second report was by Wu and Fang (2010), speculating that luteolin has chymotrypsin-like and trypsin-like catalytic activities in tumor cells. In a canine tumor cell line (DH82), luteolin was highly cytotoxic without causing considerable DNA damage (Silva *et al.*, 2013). However, no studies have examined luteolin's ability to modulate topoisomerases in human cancer cells. Further studies are needed.

Heat shock protein 90

Heat shock protein 90 (Hsp90) stabilizes newly synthesized proteins and helps maintain the functional competency of several signaling transducers involved in cell growth, survival, and oncogenesis. Therefore, interest grows in Hsp90 as an important target for molecular cancer therapy (Zhang *et al.*, 2005; Beck *et al.*, 2009). In the past few years, many specific inhibitors for Hsp90 have been developed, such as geldanamycin (GA) and its derivatives. However, GA is not used clinically because of serious toxic effects in the liver and kidney (Wang *et al.*, 2006). Despite its effectiveness in clinical trials for cancer, 17-AAG (17-allylamino-17-demethoxygeldanamycin), a GA derivative, has several problems, including stability, solubility, and hepatotoxicity. Luteolin can block Hsp90 by inhibiting its association with STAT3

(Fu *et al.*, 2012). This action degrades phosphor-STAT3 (Tyr-705) and phosphor-STAT3 (Ser-727)-phosphorylated STAT3 through a proteasome-dependent pathway. Hsp90 is one of the most important regulators of the Akt signaling pathway (Zhang *et al.*, 2005; Beck *et al.*, 2009). Surprisingly, a recent study presented protein phosphatase 2A (PP2A) as an alternative target for luteolin (Ou *et al.*, 2013). This study suggests that PP2A activation may work with Hsp90 cleavage to inactivate Akt and lead to a vicious caspase-dependent apoptotic cycle.

Stabilization of tumor suppressor protein p53

The tumor suppressor protein p53, a transcription factor, controls the cell cycle (and arrests it in case of DNA damage). Inhibition of tumor growth through cell cycle arrest and induction of apoptosis are functionally related to p53 (Kobayashi *et al.*, 2002; Didelot *et al.*, 2003). Luteolin could mediate p53 stabilization and accumulation, which induces apoptosis and prevents cell proliferation in many cancer cell lines, including breast cancer (Momtazi-Borojeni *et al.*, 2013), Eca109 (Wang *et al.*, 2012b), gastric cancer AGS (Wu *et al.*, 2008), HT-29 colon cancer (Lim do *et al.*, 2007), and head and neck and lung cancer (Amin *et al.*, 2010). In two human colorectal carcinoma-derived cell lines with microsatellite instability – CO115 with wild-type p53 and HCT15 harboring a p53 mutation – luteolin enhanced p53 expression (Xavier *et al.*, 2011). In an in-vivo nude mouse xenograft model, luteolin enhanced cisplatin's anticancer activity by promoting p53 stabilization and accumulation (Shi *et al.*, 2007). Also, luteolin ameliorates cisplatin's nephrotoxicity by downregulating the p53-dependent apoptotic pathway in the kidney (Kang *et al.*, 2011).

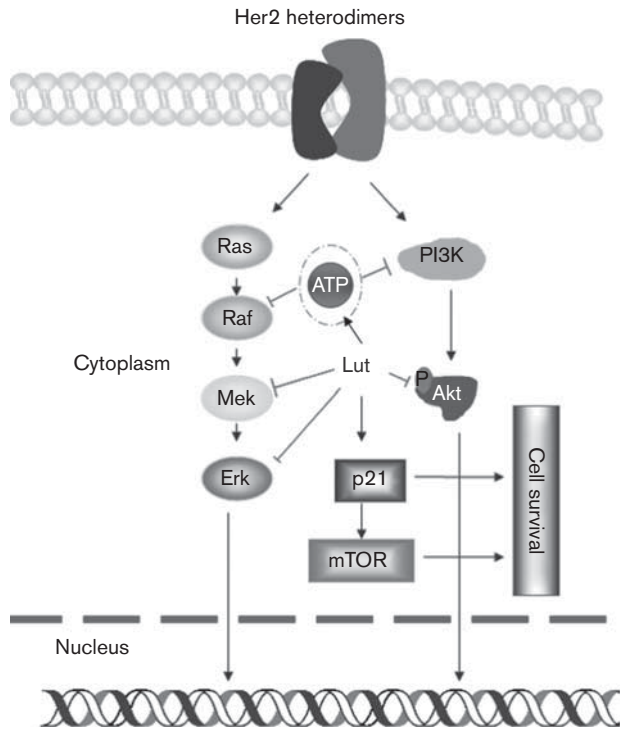
Mammalian target of rapamycin signaling

Mammalian target of rapamycin (mTOR), a key regulator of various cellular activities, belongs to the family of PI3K-related kinases and is one of the most commonly activated signaling pathways in human cancer (Faivre *et al.*, 2006). Chiang *et al.* (2007) showed that luteolin inhibited cell proliferation and mediated apoptosis in HER2-overexpressing cancer cells. Also, in nude mice with xenografted SKOV3.ip1-induced tumors, luteolin inhibited HER2 expression and tumor growth. In that study, but only at low doses, luteolin upregulated the expression of p21 and transiently inhibited mTOR signaling. That finding suggests luteolin's inability to cause sustained Akt/mTOR inhibition, which may contribute to the p21 induction that may confer a survival advantage on HER2-overexpressing cancer cells (Fig. 4). Therefore, suppressing p21 expression along with mTOR inhibition may be a good way to improve anticancer drugs against HER2-overexpressing tumors.

Raf and PI3K

KRAS and BRAF mutations are common in colorectal carcinoma and can activate proliferation and survival

Fig. 4



Luteolin (Lut) modulates the Raf/PI3K signaling pathway in KRAS and BRAF mutated cancer cells and HER2-overexpressing cancer cells. Luteolin inhibits Ras's downstream client proteins and PI3K signaling pathways. Luteolin noncompetitively binds with ATP to abolish Raf activity and competitively binds with ATP to inhibit PI3K activity. However, p21 induction by luteolin could confer on cancer cells a survival advantage by activating mTOR signaling. mTOR, mammalian target of rapamycin.

through MAPK/ERK and/or PI3K signaling pathways. In KRAS-mutated HCT15 cells, luteolin decreased ERK phosphorylation, whereas it had no effect on phospho-ERK in BRAF-mutated CO115 cells. This finding suggests that luteolin's antiproliferative and apoptotic effects can be attributed to its activity on KRAS and PI3K, but not on BRAF (Xavier *et al.*, 2009). In another study, luteolin inhibited Raf and PI3K activities and attenuated phosphorylation of MEK and Akt (Kim *et al.*, 2013). The potential mechanism for this event is that luteolin non-competitively binds with ATP to suppress Raf activity and competitively binds with ATP to inhibit PI3K activity (Fig. 4).

Preventing tumor invasion and metastasis

Metastasis is the major cause of death from cancer (Weng and Yen, 2012; Lin *et al.*, 2013). In metastasis, cancer cells migrate from the primary tumor to other sites, forming secondary tumors. Several reports showed that flavonoids naturally inhibit cancer invasion and metastasis. As discussed above, studies have confirmed luteolin's antiproliferative activities in many cancer cell lines, but how

it affects invasion by cancer cells remains unclear. Figure 5 shows the possible molecular targets whereby luteolin inhibits the invasion of cancer cells.

Integrin $\beta 1$ and focal adhesion kinase

Hypoxia-induced epithelial-mesenchymal transition (EMT) is an essential step in cancer metastasis. Luteolin inhibits the expression of integrin $\beta 1$ and focal adhesion kinase (FAK), which are closely related to EMT formation. This relationship suggests that luteolin inhibits hypoxia-induced EMT, at least in part, by inhibiting the expression of integrin $\beta 1$ and FAK (Ruan *et al.*, 2012a). Luteolin also inhibits EMT in malignant melanoma cells both *in vitro* and *in vivo* by regulating $\beta 3$ integrin (Ruan *et al.*, 2012b). Taken together, these findings show luteolin's potential as an anticancer chemopreventive and chemotherapeutic agent to prevent EMT.

Cycle 42

A recent study showed that luteolin prevents the migration of glioblastoma cells by affecting PI3K/AKT activation, modulating the expression of cell division protein cycle 42 (Cdc42), and facilitating its degradation by the proteasome pathway (Cheng *et al.*, 2013). This finding suggests that pharmacological inhibition of migration by luteolin is likely to preferentially facilitate the degradation of Cdc42. Understanding Cdc42's function and degradation by specific inhibitors adds another dimension for the development of potent therapeutic modalities in the context of invasion and metastasis and may be useful for cancer patients.

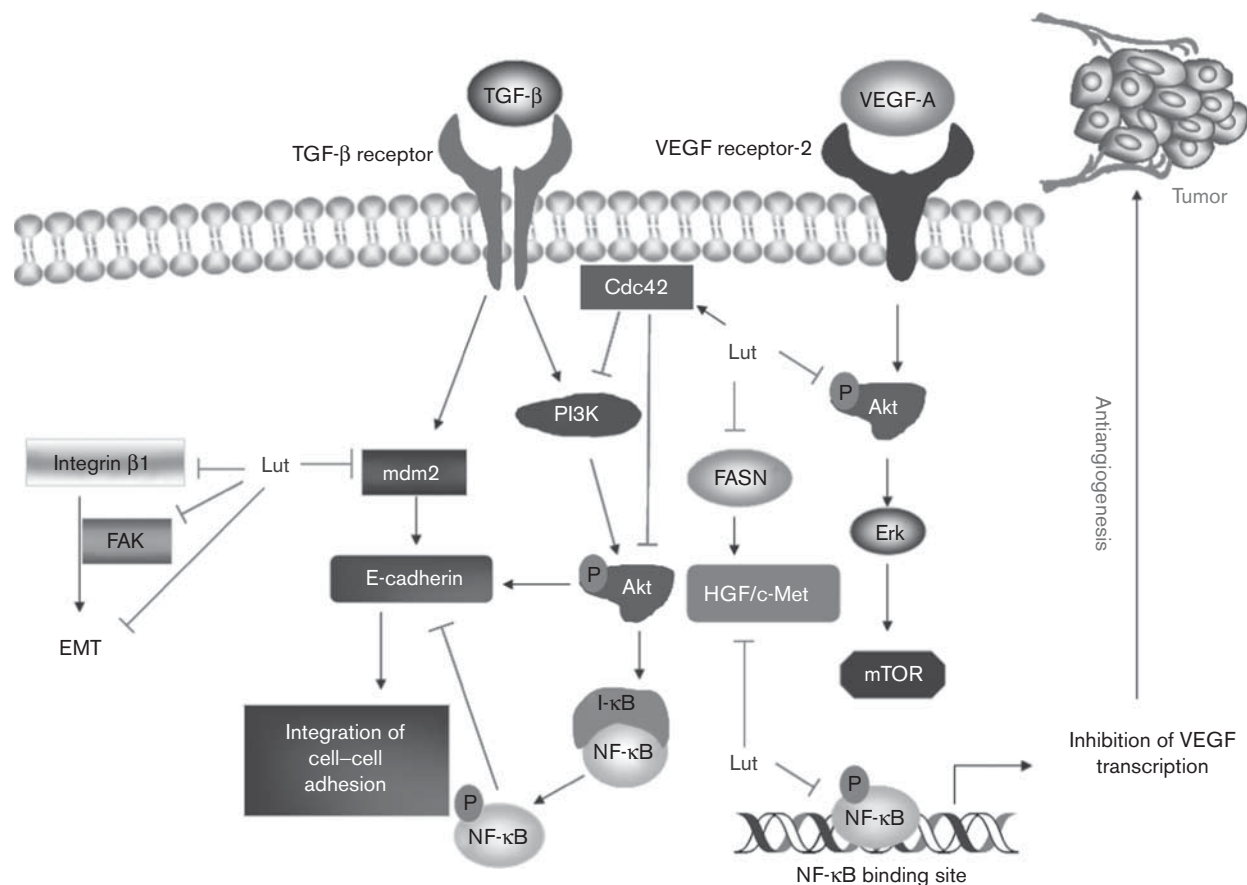
Fatty acid synthesis

Fatty acid synthesis is now associated with clinically aggressive tumor behavior and tumor cell growth and has become a novel target pathway for chemotherapy development (Cheng *et al.*, 2014; Hamada *et al.*, 2014). Coleman *et al.* (2009) reported a novel connection between fatty acid synthesis activity and c-Met protein expression, suggesting that luteolin could act as a novel hepatocyte growth factor (HGF)/c-Met inhibitor by reducing the expression of this receptor. However, adding palmitate prevented luteolin from suppressing c-Met protein expression.

c-Met tyrosine kinase

c-Met tyrosine kinase plays paramount roles in cancer invasion and metastasis in many types of cancer cells. c-Met tyrosine kinase acts as a membrane receptor for HGF. Aberrant activation of the HGF/MET signaling is strongly implicated in the malignant transformation and progression of many tumors which are characterized by an aggressive metastatic phenotype and a poor prognosis (Hack *et al.*, 2014; Lee *et al.*, 2014; Vigna and Comoglio, 2014). Luteolin acts as a novel HGF/c-Met inhibitor by suppressing phosphorylation of c-Met tyrosine kinase. Luteolin thus inhibits HGF-induced cell invasion in

Fig. 5



Possible molecular targets whereby luteolin (Lut) inhibits the invasion of cancer cells. Luteolin inhibits hypoxia-induced EMT, at least in part, by inhibiting the expression of integrin $\beta 1$ and FAK. Luteolin prevents cancer cell migration by activating the modulator protein of cell division, Cdc42, which modulates PI3K/AKT activity by facilitating its degradation through the proteasomal pathway. Luteolin acts as a novel HGF/c-Met inhibitor by suppressing phosphorylation of c-Met tyrosine kinase, induced by HGF, thereby inhibiting cancer cell invasion. Because it can reduce AKT phosphorylation, luteolin mediates inhibition of mdm2, upregulating E-cadherin. Of note, downregulation of E-cadherin results in the loss of cell-cell adhesion. Luteolin could interfere in the PI3K-Akt-NF- κ B-Snail pathway, thus attenuating TGF- $\beta 1$ -induced EMT of cancer cells. Luteolin reduces the expression of VEGF mRNA by inhibiting NF- κ B transcription activity, thus inhibiting VEGF secretion. Furthermore, luteolin suppresses VEGF-A-induced phosphorylation of VEGF receptor 2 and their downstream protein kinases AKT, ERK, and mTOR, thus reducing cell viability and possibly leading to apoptosis. Cdc42, cycle 42; EMT, epithelial-mesenchymal transition; ERK, extracellular signal-regulated kinase; FAK, focal adhesion kinase; FASN, fatty acid synthesis; HGF, hepatocyte growth factor; ; mdm2, mouse double minute 2 homolog; mTOR, mammalian target of rapamycin; NF- κ B, nuclear factor- κ B; TGF-1, transforming growth factor-1; VEGF, vascular endothelial growth factor.

human DU145 prostate and hepatoma HepG2 cancer cells (Lee *et al.*, 2006; Coleman *et al.*, 2009). Luteolin's inhibition of HGF/ME/T signaling represents a validated and effective therapeutic tool in the battle against cancer.

E-cadherin

E-cadherin, a marker of epithelial cells, maintains cell-cell adhesion. Decreased expression of E-cadherin thus leads to a prominent increase of cell invasion (Borchers *et al.*, 1997; Soncin *et al.*, 2009; Chen *et al.*, 2010; Lin *et al.*, 2011).

Luteolin prevents the invasion of prostate cancer PC3 cells by inhibiting mdm2 expression and inducing E-cadherin expression (Zhou *et al.*, 2009). Moreover, pretreatment of A549 lung cancer cells with luteolin prevented TGF- $\beta 1$

from downregulating E-cadherin, maintained normal morphological appearance, and prevented EMT of lung cancer cells (Chen *et al.*, 2013). Furthermore, TGF- $\beta 1$'s activation of the PI3K-Akt-I κ B α -NF- κ B-Snail pathway reduced the activity of E-cadherin, which pretreatment with luteolin prevented. This finding suggests that luteolin could be involved as a juvenile agent with chemotherapeutics to prevent EMT of a wide spectrum of cancer cells.

Angiogenesis

Angiogenesis, the formation of new blood vessels from existing vascular beds, plays a marginal role in tumor growth, invasion, and metastasis. Luteolin exerted strong antiangiogenesis activity in chick chorioallantoic membrane and anti-invasive activity on breast cancer cells.

It also downregulates the expression of astrocyte elevated gene 1 (AEG-1), a novel oncoprotein, and matrix metalloproteinase-2 (MMP-2) (Jiang *et al.*, 2013). Luteolin can inhibit the in-vivo growth of gastric tumors; this mechanism may correlate with downregulated expression of VEGF-A and MMP-9 (Lu *et al.*, 2013). In prostate cancer cells, luteolin suppressed VEGF-A-induced phosphorylation of VEGF receptor 2 and their downstream protein kinases AKT, ERK, and mTOR, reducing cell viability, followed by induction of apoptosis (Pratheeshkumar *et al.*, 2012). Alternatively, luteolin can reduce the expression of VEGF mRNA by inhibiting NF- κ B transcription activity, inhibiting VEGF secretion in pancreatic carcinoma cells (Cai *et al.*, 2012).

Luteolin with other anticancer drugs

MDR is an obstacle in cancer treatment, often because less drug accumulates in tumor cells owing to enhanced drug efflux (Limtrakul *et al.*, 2004). In oxaliplatin-resistant cell lines, luteolin inhibited the Nrf2 pathway and reversed MDR (Chian *et al.*, 2014a). Furthermore, in non-small-cell lung cancer, luteolin inhibits the Nrf2 pathway *in vivo* and can serve as an adjuvant in chemotherapy (Chian *et al.*, 2014b). Pretreatment of BxPC-3 human pancreatic cancer with luteolin, followed by gemcitabine inhibited protein expression of nuclear GSK-3 β and NF- κ B p65, was accompanied by increased proapoptotic cytosolic cytochrome *c* (Johnson and Gonzalez de Mejia, 2013). Coadministration of luteolin and paclitaxel activated caspase-8 and -3 and increased expression of Fas by blocking STAT3 (Yang *et al.*, 2014). In an in-vivo nude mouse xenograft model, luteolin enhanced p53 accumulation, reinforcing cisplatin's therapeutic activity (Shi *et al.*, 2007). Surprisingly, luteolin prevented cisplatin from causing nephrotoxicity by downregulating the p53-dependent apoptotic pathway in the kidney (Kang *et al.*, 2011). Finally, luteolin may act against metastasis because it can suppress the production of MMP-9 and MMP-2 and upregulate *TIMP2* gene expression (Pandurangan *et al.*, 2014). Taken together, these findings show that luteolin can serve as an adjuvant – not only to enhance the potency of chemotherapeutics but also to reduce their cytotoxicity.

Epigenetic regulation

In recent years, researchers have extensively documented that epigenetic mechanisms such as DNA methylation and histone modification regulate activities of many cancer cells (Mirza *et al.*, 2013; Yu *et al.*, 2013; Farkas *et al.*, 2014). Therefore, epigenetic regulation is an attractive target for cancer therapeutics (Ptak and Petronis, 2008). In fact, the human genome has four DNA methyltransferase genes (*DNMT*), encoding proteins with distinct functions (Mirza *et al.*, 2013). However, histone tails (and their modifications) regulate diverse biological processes such as transcription, DNA repair, cell division, and differentiation (Van Attikum and

Gasser, 2005; Duncan *et al.*, 2008). Unfortunately, the literature offers no precise information on the epigenetic regulation of luteolin in cancer cells. In a study on the HeLa cell line, luteolin-induced E3 ubiquitin-protein ligase UHRF1 and DNMT1 downregulation was accompanied by global DNA hypomethylation (Krifa *et al.*, 2013). Attoub *et al.* (2011) first presented luteolin as a potent histone deacetylase (HDAC) inhibitor that enhances cisplatin cytotoxicity in LNM35 cells and reduces the growth of LNM35 tumor xenografts in athymic mice (Attoub *et al.*, 2011). However, an urgent need remains to study epigenetic regulation of luteolin in different cancer cell lines. By taking advantage of epigenetic modifications, we can use HDAC and DNMT inhibitors to control various cancer cell activities. Moreover, luteolin may be a promising HDAC inhibitor for cancer treatment. The US Food and Drug Administration has already approved some HDAC and DNMT inhibitors, such as azanucleoside drugs, to treat myelodysplastic syndromes and acute myeloid leukemia (Garcia-Manero and Fenaux, 2011; Yu *et al.*, 2013).

Conclusion

Luteolin is a potent anticancer agent that could halt a wide spectrum of tumors and cancer cells, including MDR cells. Preclinical and clinical trials using luteolin as an adjuvant supplement for cancer therapy should place this fascinating agent at the forefront of new therapeutic approaches and then translate this study's concepts into clinical applications.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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