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Review

Effects of apigenin on gastric cancer cells

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ABSTRACT

Gastric Cancer (GC) is one of the most prevalent cancers worldwide. As the currently available therapeutic options are invasive, new and more benign options are being explored. One of which is Apigenin (Api), a natural flavonoid found in fruits and vegetables, such as celery, parsley, garlic, bell pepper and chamomile tea. Api has known anti-inflammatory, -oxidant, and -proliferative proprieties in several diseases and its potential as an anticancer compound has been explored. Here we systematize the available data regarding the effects of Api on GC cells, in terms of cell proliferation, apoptosis, Helicobacter pylori (H. pylori) infection, and molecular targets. From the literature it is possible to conclude that Api inhibits cell growth in a dose- and time-dependent manner, which is accompanied by the reduction of clone formation and induction of apoptosis. This occurs through the Akt/Bad/Bcl2/Bax axis that activates the mitochondrial pathway of apoptosis, resulting in restriction of cell proliferation. Additionally, it seems that the anti-proliferative potential of Api on GC cells is particularly relevant in a more aggressive GC phenotype but can also affect normal gastric cells. This indicate that this flavonoid must be used in low-to-moderate doses to avoid side-effects induced by disturbance of the normal epithelium. In H. Pylori-infected cells, the literature demonstrates that Api reduces inflammation by diminishing the levels of H. pylori colonization, by preventing NF-kB activation and by diminishing the production of reactive oxygen specimens (ROS). Accordingly, in GC Api seems to regulate different hallmarks of cancer, such as cell proliferation, apoptosis, cell migration, inflammation and oxidative stress, demonstrating its potential has an anti-GC compound.

1. Introduction

Gastric Cancer (GC) is one of the most prevalent tumours worldwide, being the second mostly deadly cancer [23,40]. GC is asymptomatic or has nonspecific symptoms in early stages of the disease and when the symptoms are apparent it is usually already in an advanced stage which translates in a worse prognosis [8]. Treatment of GC is based on surgery for total or partial removal of the affected area, when it is still restricted to the gastric mucosa – early stages – and chemotherapy for more advanced stages, both of which are quite invasive and aggressive procedures for the patient [31]. Accordingly, over the last few years, efforts have been made to develop more suitable therapeutic strategies and new doors have been opened with the study of natural compounds with potential anticancer effects. One such compound is apigenin (Api), known chemically as 4',5,7-trihydroxyflavone, that is one of the most common flavonoids, and can be found in fruits and vegetables, with particular abundance in celery, parsley, garlic, bell pepper and chamomile tea [16]. Api has a wide range of pharmacological properties and has been used in traditional medicine for centuries, mainly due to its anti-inflammatory, -oxidant, -toxic, -bacterial, -viral, -parasitic, -fungal, -diabetic, -allergic and hemostatic properties [36,52]. Chamomile, which is one of the highest sources of Api, has been reported to be effective in relieving gastritis symptoms and is used as an inhalant vapor that reduces inflammation, and in skin care products [48]. Several pharmacokinetic studies established that Api reverses the adverse effects of cyclosporine-induced kidney damage, exerted immunomodulatory effects against rheumatoid arthritis and other autoimmune diseases [1]. Api has also been shown to have protective effects against Alzheimer

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and Parkinson diseases, as well as ischemic reperfusion injury, cardiovascular disease, amnesia. Additionally, this flavone has beneficial effects in cases of depression and insomnia [2,11,14,19,21,27,45,47,51,55,57]. The first study on the anti-mutagenic effects of Api was carried out by Birt et al. in 1986 and since then the anticancer and anti-proliferative effects, as well as the pathways affected by the Api, have gained interest from the scientific community [4]. More recently, it has been proven that Api is an effective compound in tumour suppression of several types of cancers, namely colorectal, breast, prostate, oral and liver cancer, as well as some types of leukaemia [9,20,35,38,46,53]. The evolution of any type of neoplasia is a complex process that involves a series of genetic and epigenetic alterations that lead to the initiation, promotion and cancer progression [12]. These alterations consist of over- or under-expression of proteins that regulate different biological processes, usually in processes such as proliferation, differentiation and survival [12]. With the various studies carried out to date, it is known that Api inhibits cell growth and proliferation, promotes apoptosis, induces cell cycle arrest and autophagy and is able to disrupt the mitochondrial membrane potential in vivo and in vitro [8,18,25,50,54]. Nevertheless, there is no systematization of the mode of action of Api in GC: Accordingly, here we review the studies addressing the role of Api on GC in terms of cell proliferation, apoptosis, Helicobacter pylori (H. pylori) infection and molecular targets.

2. Methods

2.1. Review design

In this systematic review, the PRISMA System [30] was used to select publications that reported the effects of Api on GC cells. Using the PRISMA Guidelines [30], published literature on the effects of Api on GC cells was systematically searched in the Scopus, PubMed and Web of Science databases. To maximize the search and reduce selection biases, the search was restricted to articles in English, using keywords such as: "apigenin AND gastric cancer" and "flavonoids AND gastric cancer". No restrictions were considered regarding the date of execution or publication of the articles, until September 2023. During the initial search, all articles identified in the indexed databases (Scopus, Pubmed and Web of Science) were first selected based on their titles and abstracts. All authors carried out the research, independently, in the mentioned databases and removed articles according to the eligibility criteria. All articles were subject to a primary review, where the titles and abstracts of each article were analyzed. From this research and primary review, the articles were divided into three categories: "relevant articles", "non-relevant articles" and "uncertainties". Lists were compared by all authors and articles in duplicate or triplicate were excluded. These three categories were discussed by all authors, who made the final selection of articles to be included. Any disagreement or non-compliance was discussed until a consensus was reached. Each article on the final list of articles to be included was analyzed and discussed in its entirety, with the following variables removed: name of the first author, year of publication, objective of the work, methodology used, type and size of sample, treatment conditions with Api, effects of Api on the sample under study, effects of Api on GC and molecular targets of Api. Information extraction followed the PRISMA Guidelines [30].

2.2. Inclusion and exclusion criteria

Only articles describing the association of Api with GC cells were included in this review. All sources of information and unverified studies that did not meet these criteria were excluded.

2.3. Selection of articles

After pooling different publications from the searched databases, all those not written in English were excluded, as well as all publications that reported the effects of Api on other types of cancer. The risk of bias was reduced by including only published articles and excluding studies whose research methodology was not understandable.

3. Results and discussion

3.1. Articles included in the review

Through the PRISMA 2020 diagram, we demonstrate the selection criteria used to select articles for this review. This diagram is represented in Fig. 1. A total of 237 articles were retrieved from the indexed databases. All articles were listed on an Excel sheet and 29 articles in duplicate or triplicate were excluded and two articles were excluded for other reasons, leaving 206 articles. A more extensive analysis by title and abstract of the articles was performed, and 35 articles were excluded, leaving 171 articles. Of these, after a full evaluation, 165 articles were excluded, leaving six articles evaluated according to the eligibility criteria and eligible for inclusion in this review.

Of the 242 initial articles, six met the eligibility criteria and were included in this review. In the present review, two studies directly related the effects of API on GC [8,50] One of the six articles related the effects of API with GC induced by infection with H. pylori [18], while another related the antiproliferative activities of flavonoids from the D. kotschyi plant with human gastric adenocarcinoma cell lines [25]. Another study related the effects of API with gastric adenocarcinoma cells infected with H. pylori [49]. Finally, a study related the anti-proliferative and pro-apoptotic effects of Api with the regulation of Akt signaling pathway in gastric cancer cells [54]. The results obtained are described in Table 1.

3.2. Apigenin and effects on gastric cancer cells

3.2.1. API effects on cell proliferation

A key feature in carcinogenesis is the continuous and deregulated proliferation of cells, resulting from the absence of an appropriate response to signals that regulate cell grow and division [12]. Accordingly, cell proliferation is a common target of potential anti-cancer agents. For Api, the effects on proliferation of GC cells were assessed by some authors as presented in Table 1. In the first study, performed by [50], the GC cell line SGC-7901 was used and by testing different concentrations of Api throughout 7 days, the authors demonstrated that Api inhibited cell growth and that this effect increased in a dose- and time-dependent manner [50]. Additionally, in these settings, Api resulted in a reduction of the clone formation efficiency of SGC-7901 cells [50]. This was an important finding as the clone-forming ability of cells reflects the proliferative capacity of cells and is an indicator of undifferentiated cancer stem cell [33]. Hence, these results indicate that Api has the potential to be used as an anticancer therapeutic agent in GC cells. In another study from 2007, performed by Yuan et al., it was also concluded that Api has anti-proliferative effects on SGC-7901. In this study, cells were treated with 0, 2.5, 5, 10, 20, 40 and 80 μmol/L of Api in an interval of 1 to 7 days. The effects of Api were evaluated through the MTT assay, and results demonstrated that cell proliferation is refractory to low concentrations of Api (2.5, 5 and 10 μmol/L), whereas it is inhibited with 20- 80 μmol/L of Api [54]. Similarly, [25], by studying the effects of different compounds extracted from the D. kotschy plant (Iranian medicinal plant) on cell lines derived from a variety of cancer types, determined that Api presents anti-proliferative properties against the gastric adenocarcinoma cells AGS, with an IC50 of 5.1 ± 0.8 [25]. Interestingly, this flavonoid showed anti-proliferative activity towards all tested cell lines: human acute promyelocytic leukemia (HL-60), human colon carcinoma (HT-29), human osteosarcoma (SaOs-2), murine fibrosarcoma (WEHI-164) and human fetal foreskin fibroblast (HFFF-P16). Nevertheless the more pronounced anti-proliferative effect was the observed in AGS [25]. These data suggest that Api has a strong anti-proliferative potential in GC but can also be explored in other

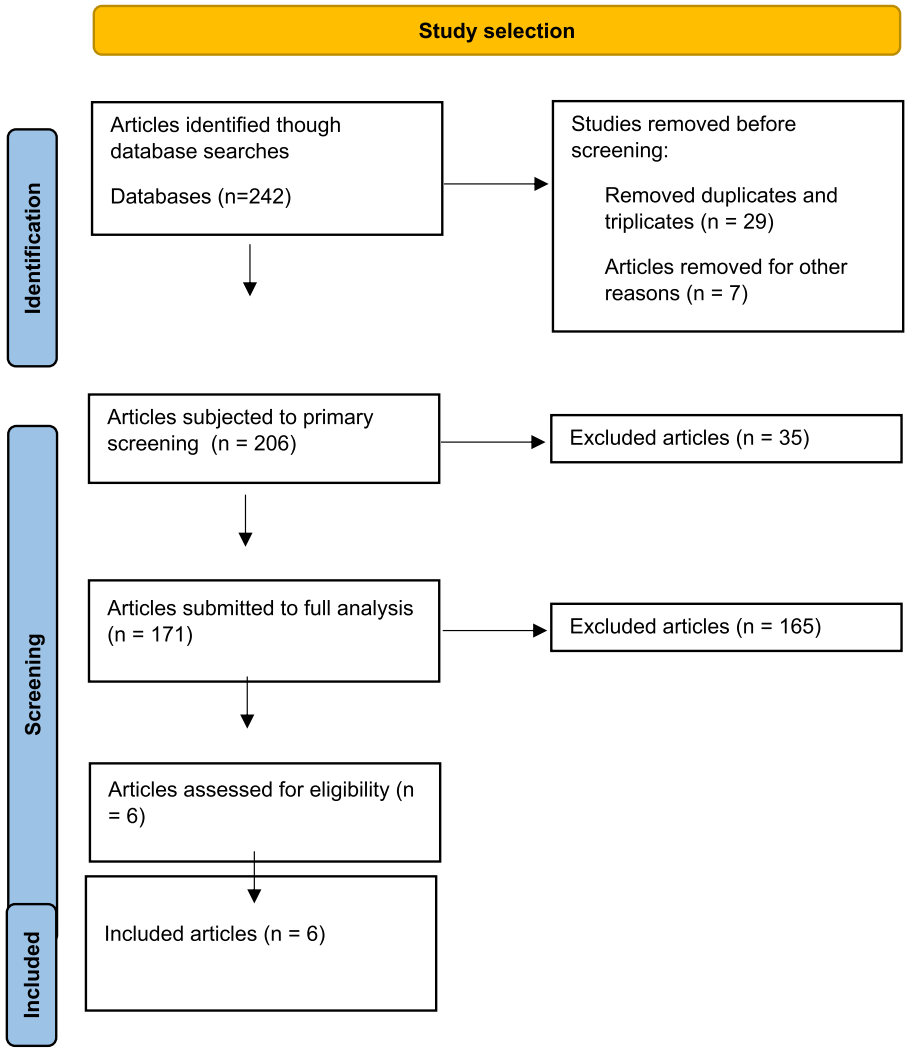

Fig. 1. PRISMA 2020 flow diagram for new systematic reviews which included searches of databases, taken from Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71.

human tumors. A more recent study, carried out by [8], also proved that Api have anti-proliferative effects on GC cells [8]. This study demonstrated that the inhibitory effects of Api on cell proliferation occur in a time and dose-dependent manner, and that this inhibition is more evident in undifferentiated (HGC-27) cells than in a semidifferentiated cell line (SCG-7901) [8]. Of note, the authors also demonstrated that Api did not cause any change in GES1, a cell line from the normal gastric epithelium, except when cells were exposed to high concentrations of this compound (20 μg/mL). Accordingly, this study suggests that Api has an anti-proliferative potential on GC cells, particularly with a more aggressive phenotype, but also indicates that this flavonoid must be used in low-to-moderate doses to avoid side-effects induced by disturbance of the normal epithelium.

Collectively, these studies suggest that Api has the potential to be used as an anticancer therapeutic agent in GC, due to its anti-proliferative ability (Fig. 2). Although the limited studies, some observations can be pointed out, such as the fact that Api seems to be more effective in a more aggressive phenotype of the disease, as it presents a more pronounced anti-proliferative activity in undifferentiated versus semidifferentiated cells [8]. Additionally, the fact that the growth of normal gastric epithelium cells seems to be also affected by Api [8] indicates that the dose of this compound needs to be carefully determined, to avoid adverse secondary effects. Nevertheless, as this flavonoid seems to act in a time-dependent manner, the choice of dose can be adjusted with the time of exposure.

3.3. *Effects of API on apoptosis*

Apoptosis is a way of cell death comprising a dramatic set of perturbations at the nuclear and cellular level that culminates in cell demise and cleaning by phagocytosis [44]. Many anti-cancer agents act by inducing apoptosis and, in this regard, three of the studies included in this review addressed the apoptotic effects of Api on GC cells. The obtained results demonstrated that Api induces cell shrinkage, cell crumple, apoptotic bodies and margination, loss of cell-cell contact and cell transparency, as well as anisokaryosis, karyorrhexis, chromatin condensation, loss of definition of the nucleolus and caspase-3 cleavage [8,50,54], all typical features of apoptosis [44]. These alterations were observed in the GC cell lines SGC-7901 and HGC-27, in a dose-dependent fashion, whereas did not occurred in normal GES1 cells [8,50,54]. By comparing the anti-apoptotic effects of Api on SGC-7901 and HGC-27 cells, it was observed that Api increased the rate of apoptosis in 14.2% and about 50% for primary and advanced stages of apoptosis, respectively, in HGC-27 cells; whereas SCG-7901 cells experienced an increase in apoptosis from 3.32 to 37.4% for primary apoptosis, and from 2.24 to 11.1% for later apoptosis. In total, the apoptosis rates were 64.2% and 48.5% for HGC-27 and SCG-7901 cells, respectively [8]. The predominance of advanced stages of apoptosis and

Table 1
Summary of the results of the included studies.

Sample	Treatment conditions with apigenin	Effects on proliferation	Effects on apoptosis	Effects on cell cycle	Effects on H. pylori infection	Molecular targets	Reference
GC Cell Line (SGC-7901)	**Proliferation:** 0, 20,40 and 80 μmol/L, 7 d **Apoptosis:** 0, 20, 40 and 80 mmol/L /24 h and 48 h	Growth inhibition rate of 38%, 71% e 99% for 20,40 and 80 μmol/L at day 7.	Decrease of cell transparency, increase of cell crumple, cleared cell boundaries, karyorrhexis, and nucleolus not obvious. Apoptosis rate: 5.76%, 19,17% and 29,30% for 20, 40 and 80 mmol/L 48 h, respectively.	Accumulation in the S phase.	N.A.	N.A.	Wu et al., [50]
GC Cell Line (SGC-7901)	**Proliferation**: 0, 2.5, 5, 10, 20, 40 e 80 μmol/L for 1 to 7 days; **Apoptosis:** 60 μmol/ L por 12, 24 e 48 h	Inhibition in a dose dependent-manner: > 20 μmol/L	Loss of cell-cell contact, cell shrinkage, chromatin condensation, and caspase-3 cleavage.	N.A.	N.A.	Inhibition of Akt with dephosphrylation of Bad	Yuan et al., [54]
GC Cell Lines HGC-27 and SGC-7901	**Proliferation:** 0, 1, 2.5, 5, 10 e 20 μg/ mL for 24 h, 48 h and 72 h. **Apoptosis:** 0, 10 and 20 μmol/L por 24 h e 0 e 10 μmol/ L for 48 h; **Cell cycle:** 0 and 10 μmol/ L for 24 h	Inhibition in a time- and dose-dependent manner, with a more pronounced effect on undifferentiated HGC-27 than in semidifferentiated SGC-7901 cells.	Nuclear alterations typical from apoptosis. Increased rate of apoptosis: 14.2% and 37.4% of early apoptosis and 50% and 11.1% for late apoptosis for HGC-27 and SGC-7901.	No changes	N.A.	Decrease in mitochondria membrane potential, especially in HGC-27 cells. Decrease in expression of Bcl-2 and increase of Bax and caspase-3	Chen et al., [8]
GS Cell Line AGS	**Proliferation:** various concentrations for 72 h	Inhibition, with an IC_{50} 5.1 ± 0.8 μg/mL	N.A	N.A.	N.A.	N.A.	Moghaddam et al.,[25]
GS cell line (MKN45)	9,3 - 74 μM	N.A.	N.A.	N.A.	Anti-inflammatory action	Increase in expression of IκBα, with inhibition of nuclear factor kappa B (NF-κB). Decrease in expression of COX-2, ICAM-1, IL-6, and IL-8) and increase of MUC-2. Decrease of ROS levels.	Wang, Huang [49]
Mongolian gerbils	30-60 mg/kgbw/ day	N.A.	N.A.	N.A.	Decreased atrophic gastritis and dysplasia/GC. 60 mg/kgbw/day: decreased H. pylori colonization and histological changes of neutrophil and monocyte infiltrations and atrophic gastritis.	N.A.	Kuo et al., [18]

higher overall rate of apoptosis in undifferentiated HGC-27 cells comparing to the observed in semidifferentiated SCG-7901 accompanied the tendency of proliferation inhibition, which suggests that, at least in these settings, Api reduces cell proliferation by inducing cell death.

Collectively, these observations indicate that Api induces apoptosis in a dose-dependent fashion, and that this induction is more pronounced on undifferentiated versus semidifferentiated GC cells. This indicates that Api might be more effective at more aggressive cases of GC. Additionally, as this effect mirrors the anti-proliferative ability of Api, one might speculate that Api reduces GC proliferation by inducing apoptosis (Fig. 2).

3.4. API effects on Helicobacter pylori infection

H. pylori infection can cause inflammation of the gastric epithelium that can progress to atrophic gastritis, intestinal metaplasia and even gastric adenocarcinoma [15]. As Api has known anti-inflammatory effects in different conditions, it is of interest to determine the effects of this flavonoid in the infection by H. pylori. In line with this, two different studies addressed the anti-inflammatory effects of Api on gastric cancer cells infected with Helicobacter pylori (H. pylori).

[18] studied the effects of Api on H. pylori-induced atrophic gastritis and gastric cancer progression in vivo [18]. In this study, the authors inoculated H. pylori in Mongolian gerbils with atrophic gastritis or gastric cancer and addressed the histological changes of bacteria colonization, neutrophil and monocyte infiltrations, as well as atrophic gastritis in both groups. Results demonstrated that Api decreases H.

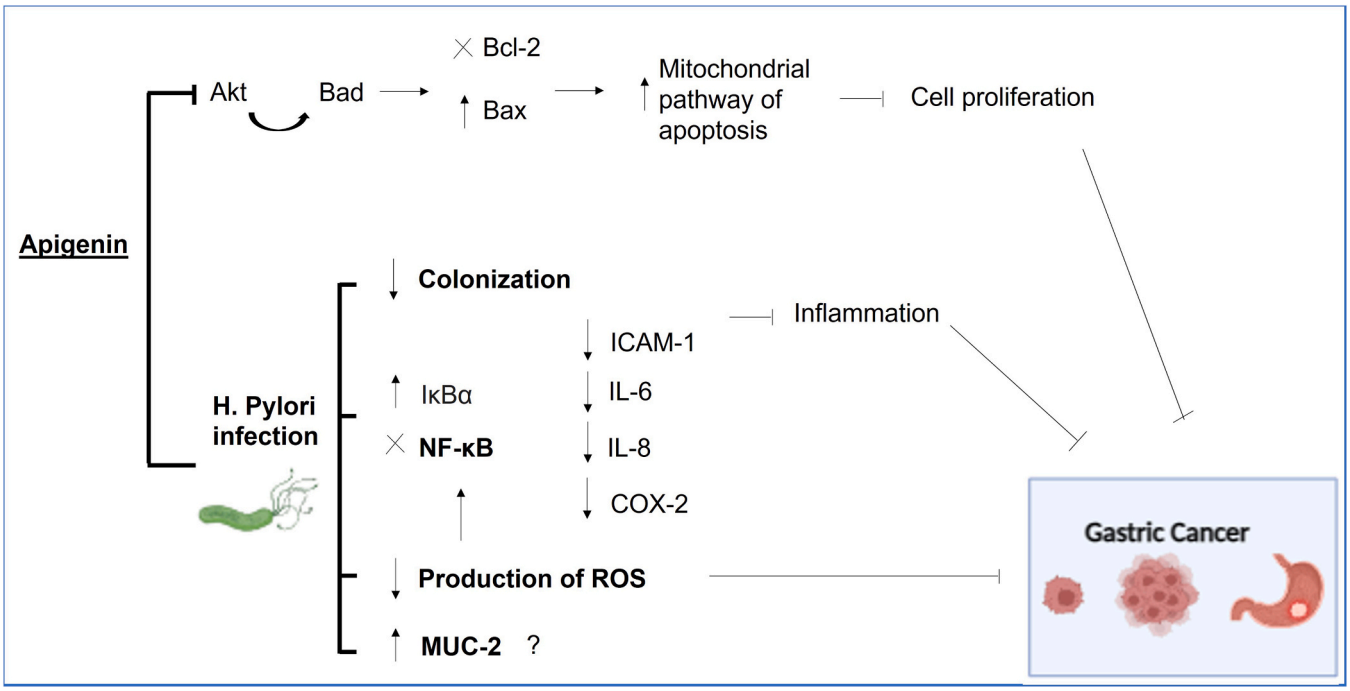

Fig. 2. 9 Apigenin restrains gastric cancer development and growth. 1-Apigenin (Api) inhibits gastric cancer (GC) cell proliferation through induction of apoptosis. Api induces the mitochondrial pathway of apoptosis by inhibiting Akt by dephosphorylation which results in inhibition of Bad and reduction of Bcl-2. The pro-apoptotic Bax is activated, which results in caspase-3 activation and concomitant apoptosis. 2) Upon Helicobacter Pilory (H. Pylori) infection, apigenin diminishes the detectable levels of H. Pylori at the stomach and counteracts H. Pylori-induced inflammation by increasing the IκBα expression which results in NF-κB inactivation and concomitant decrease in the levels of the inflammatory factors ICAM-1, IL-6, IL-8 and COX-2. In addition, Api restrains the production of reactive oxygen specimens (ROS), that activates NF-kB and might induce extensive gastric mucous damage and progression into intestinal metaplasia and GC. ROS also contributes to the development of GC through induction of genotoxicity, DNA damage, metabolic adaptation, drug resistance and cell death. In addition, the expression of Mucin-2 (MUC-2), a secreted and gel-forming mucin, is induced by Api in H. pylori-infected cells. Although it is known that MUC-2 contributes to the formation of the mucus barrier, thus having a protective effect of the mucosa, its expression has been related to worse prognosis and survival rate in GC patients, so the significance of this increase induced by Api remains to be determined.

pylori colonization and the histological changes of neutrophil and monocyte infiltrations and atrophic gastritis in both groups, with a decrease in atrophic gastritis (atrophic gastritis group) and dysplasia/GC (gastric cancer group) [18]. A similar conclusion was reached by [49] which addressed the effects of Api on inflammatory factors on H. pylori-infected MKN45 gastric adenocarcinoma cells [49]. The authors observed that Api treatment resulted in inhibition of inflammation through inactivation of NF-kB, inhibition of oxidative stress through reduction of reactive oxygen specimens (ROS) and in increase of mucin-2 (MUC-2) expression (Fig. 2) [49].

Collectively these studies demonstrate that Api treatment inhibits HP-induced inflammation by reducing HP colonization, NF-kB and ROS (Fig. 2). As inflammation and oxidative stress contributes to the development of GC, these data further confirm the potential of Api has an anti-GC compound. The molecular mechanisms involved in these processes will be discussed below.

3.5. API molecular targets

The molecular targets of Api in other diseases are starting to be elucidated. For instance, in obesity it is known that Api reduces adipogenesis by inhibiting the STAT3/CD36 axis [42,43]. In addition, in cervical cancer, it was found that this flavonoid restrains the FAK and PI3K/Akt/mTOR pathways, and inactivated or activated various signaling targets, such as Bcl-2, Bax, p21cip1, CDK1, CDC25c, cyclin B1, fibronectin, N-cadherin, vimentin, laminin, and E-cadherin [10].

For GC, [54] observed that in SGC-7901 GC cells a key target of Api was Akt and that treatment with this flavonoid resulted in dephosphorylation of Akt on Thr-308 and Ser-473, in a time-dependent manner [54] (Fig. 2). Additionally, the authors decided to address the activity of Bad, one Akt substrate whose function is regulated by the Akt-dependent phosphorylation of Ser136, and observed that Api treatment resulted in dephosphorylation of Bad [54]. As Bad assumes a pro-death function when dephosphorylated [13,34], the end result of Api treatment is apoptosis (Fig. 2). Accordingly, the authors speculated that the induction of apoptosis through the Akt-Bad axis was the way by which Api inhibited the growth of SGC-7901 GC cells [54]. Indeed, by evaluating the molecular mechanisms by which apoptosis was being induced by Api, [8] demonstrated that the mitochondria membrane potential was decreased along with the expression of Bcl-2, in opposition to Bax and caspase-3 expression that was augmented [8] (Fig. 2). Accordingly, Api might restrain GC proliferation through induction of the mitochondrial pathway of apoptosis by inhibiting the Akt kinase activity towards Bad, neutralizing the anti-apoptotic protein Bcl-2 and potentiating the pro-apoptotic Bax, which results in caspase-3 activation and concomitant apoptosis (Fig. 2).

The observation that Akt pathway might constitute a cellular target of Api has particular relevance as the PI3K/Akt/mTOR pathway has been reported to be one of the pathways that drives oncogenicity in GC [3]. Accordingly, Api might constitute an important anti-cancer agent in GC, through the inhibition of the PI3K/Akt/mTOR node. Of note, a study performed by [9] demonstrated that Api decreased the proliferation rate in cisplatin-resistant colon cancer cells through the induction of autophagy and apoptosis, and that these events occurred through targeting the mTOR/PI3K/Akt signaling pathway [9]. Additionally, by studying malignant melanoma cells, [56] demonstrated that cell growth and migration were inhibited in a dose and time-dependent manner by Api, that this occurred through induction of apoptosis and cell cycle arrest in the G2/M in a Akt/mTOR-dependent manner [56]. Accordingly, it seems that the PI3K/Akt/mTOR pathway as a widespread role in the

potential anti-cancer activity of Api.

As previously mentioned, in addition to PI3K/Akt/mTOR, Api seems to target IkBα/NF-kB signalling [49]. This observation was made in GC cells infected with H. pylori and it was determined that Api-mediated inactivation of NF-kB counteracts the inflammation caused by H. pylori [49] (Fig. 2). H. pylori induces an inflammatory response through NF-kB signalling activation which results in the production of known inflammatory factors, chemokines and cytokines, such as COX-2, ICAM-1, IL-6, and IL-8 [49]. The inflammatory signals are further augmented through the induction of ROS [49]. By treating HP-infected MKN45 cells with Api, the authors found that Api had an anti-inflammatory effect through increase of IκBα with concomitant inhibition of NF-κB activation. This resulted in a decrease of COX-2, ICAM-1, IL-6 and IL-8 levels, as well as in production of ROS, which further accentuates the anti-inflammatory role of Api, has ROS activates NF-κB [49] (Fig. 2). Similarly, another study demonstrated that Api, when administrated to mice, results in inhibition of NF-κB signaling [39]. The inactivation of NF-κB signaling was accompanied by down-regulation of genes involved in proliferation (cyclin D1, and COX-2), apoptosis (Bcl-2 and Bcl-xL), and angiogenesis (VEGF), which contributed to inhibition of prostate tumorigenesis in mice [39]. Additionally, in cells derived from pulmonary mucoepidermoid carcinoma, Api treatment altered the gene expression pattern of mucin through the regulation of NF-κB signaling [37]. Accordingly, the NF-κB signaling seems to be a target of Api, regardless of the cellular context. The role of NF-κB signaling in GC carcinogenesis is well stablished, has it regulates growth factors, cytokines, anti-apoptotic proteins, cell cycle regulators, and metalloproteinases [7]. Indeed, it has been demonstrated that NF-κB signaling is one of the most important pathways in the development of GC [28]. Accordingly, by targeting this pathway, Api might counteract the development of GC, through inhibition of NF-κB.

Mucins are glycoproteins that compose the mucus that protects the mammalian epithelia, and are responsible for its biochemical and biophysical properties [5]. The expression and distribution of mucins vary among the gastrointestinal tract and its presence determine the classification of adenocarcinoma as with gastric, intestinal, mixed or unclassified/null phenotype [17]. Among them, Mucin-2 (MUC-2) is synthesized and stored in goblet cells and is present in intestinal mucin phenotype [5]. In the study of [49], it was found that Api treatment of H. Pylori-infected cells results in increase in MUC-2 expression [49] (Fig. 2). The significance of this observation remains to be determined as the expression of MUC-2 has been associated with a worse survival rate compared to the gastric, mixed, and null mucin phenotypes [29].

Another key finding of the study performed by [49], was the fact that Api treatment resulted in reduction of ROS levels [49] (Fig. 2). Several studies have reported high oxidative stress in individuals with GC and it has been determined that this event contributes to the carcinogenesis of GC [6]. It seems that elevated ROS production induces genotoxicity, which contributes to genetic instability, DNA damage, metabolic adaptation, drug resistance and cell death [22]. Accordingly, Api might further control GC by its anti-oxidant proprieties. The ROS-mediated mechanisms of Api counteracting several types of cancers has been reviewed elsewhere [41].

Collectively, these data demonstrate that Api targets, in GC, the mitochondrial pathway of apoptosis to reduce cell proliferation and that it reduces inflammation and oxidative stress elicited by H. pylori infection. Accordingly, Api has therapeutic potential at preventing the progression of HP-induced inflammation into GC and at restraining growth of stablished tumors.

4. Conclusion

Natural products have been used as therapeutic agents for the treatment of inflammatory diseases in various parts of the world for centuries [26]. Currently, in vitro and in vivo experiments with flavonoids or other plant-derived components are being carried out, to reveal what further health benefits these phytochemicals have. Accumulated evidence suggests that Api, a natural flavonoid, has a wide range of pharmacological activities and has tremendous therapeutic value against different types of cancers [32].

In GC it seems that Api has the ability to decrease the formation of clones and inhibit cell proliferation in a dose and time-dependent manner, with a more pronouncedly effect in undifferentiated cells, without altering the normal gastric epithelium, when present in low-to-moderate doses. In addition, it seems that the growth-suppressive activity of Api in GC cells is associated, at least in part, to the induction of apoptosis. In H. pylori-infected gastric epithelium, Api decreases inflammation in vitro and decreases atrophic gastritis and GC, as well as colonization of gastric epithelium by H. pylori, in in vivo studies. In addition, it seems that Api is a multi-targeting agent as it can regulate key agents and important signaling pathways, such as Akt/Bad/Bax and IkBα/NF-kB, which are involved in the development and cancer progression, as evidenced by in vitro and in vivo studies; as well as the levels of ROS in GC cells.

One of the most important pathways in tumor progression, and one that plays an important role in the development of GC, is the mTOR signaling pathway. This pathway plays important roles in regulating cell growth, proliferation and survival and, thus, its deregulation is associated with tumorigenesis, angiogenesis, tumor growth and metastasis [24]. As mTOR integrates signals from Akt, which is a target of Api, it would be interesting to addressed whether Api targets Akt/mTOR in GC cells.

In summary, according to the studies included in this review, Api has the ability to modulate different hallmarks of cancer, such as cell proliferation, apoptosis, inflammation and oxidative stress, in the context of GC.

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CRediT authorship contribution statement

Cervantes Renata: Resources. **Mendonça Paula:** Conceptualization, Data curation, Supervision. **Pratas Ana:** Formal analysis, Methodology, Writing – original draft. **Marques-Ramos Ana:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Supervision, Validation, Writing – original draft, Writing – review & editing. **Palma Raquel:** Formal analysis, Methodology, Writing – original draft. **Malhão Beatriz:** Formal analysis, Methodology, Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

References

[1] F. Ali, Rahul, F. Naz, S. Jyoti, Y.H. Siddique, Health functionality of apigenin: a review, Int. J. Food Prop. 20 (2017) 1197–1238, https://doi.org/10.1080/10942912.2016.1207188.

[2] Z. Alibabaei, Z. Rabiei, S. Rahnama, S. Mokhtari, M. Rafieian-kopaei, Matricaria chamomilla extract demonstrates antioxidant properties against elevated rat brain oxidative status induced by amnestic dose of scopolamine, Biomed. Aging Pathol. 4 (2014) 355–360, https://doi.org/10.1016/j.biomag.2014.07.003.

[3] A. Baghery Saghchy Khorasani, A. Pourbagheri-Sigaroodi, A. Pirsalehi, A. Safaroghli-azar, M.R. Zali, D. Bashash, The PI3K/Akt/mTOR signaling pathway in gastric cancer; from oncogenic variations to the possibilities for pharmacologic interventions, Eur. J. Pharmacol. 898 (2021) 173983, https://doi.org/10.1016/j.ejphar.2021.173983.

[4] D.F. Birt, B. Walker, M.G. Tibbels, E. Bresnick, Anti-mutagenesis and anti-promotion by apigenin, robinetin and indole-3-carbinol, Carcinogenesis 7 (1986) 959–963, https://doi.org/10.1093/carcin/7.6.959.

[5] D. Boltin, Y. Niv, Mucins in gastric cancer - an update, J. Gastrointest. Dig. Syst. 3 (2013) 15519, https://doi.org/10.4172/2161-069X.1000123.

[6] M.B. Braga-Neto, D.V.S. Costa, D.M.M. Queiroz, F.S. Maciel, M.S. De Oliveira, A. B. Viana-Junior, F.A. Santos, R.F.C. Leitao, G.A.C. Brito, P.R.L. Vasconcelos, L.L.B. C. Braga, Increased oxidative stress in gastric cancer patients and their first-degree relatives: a prospective study from Northeastern Brazil, Oxid. Med. Cell. Longev. 2021 (2021) 1–9, https://doi.org/10.1155/2021/6657434.

[7] S. Chaithongyot, P. Jantaree, O. Sokolova, M. Naumann, NF-κB in gastric cancer development and therapy, Biomedicines 9 (2021) 870, https://doi.org/10.3390/biomedicines9080870.

[8] J. Chen, J. Chen, Z. Li, C. Liu, L. Yin, The apoptotic effect of apigenin on human gastric carcinoma cells through mitochondrial signal pathway, Tumor Biol. 35 (2014) 7719–7726, https://doi.org/10.1007/s13277-014-2014-x.

[9] Chen X., Xu H., Yu X., Wang X., Zhu X., Xu X. (2019) Apigenin inhibits in vitro and in vivo tumorigenesis in cisplatin-resistant colon cancer cells by inducing autophagy, programmed cell death and targeting m-TOR/PI3K/Akt signalling pathway. 6.

[10] Y.-H. Chen, J.-X. Wu, S.-F. Yang, C.-K. Yang, T.-H. Chen, Y.-H. Hsiao, Anticancer effects and molecular mechanisms of apigenin in cervical cancer cells, Cancers 14 (2022) 1824, https://doi.org/10.3390/cancers14071824.

[11] J.S. Choi, Md Nurul Islam, Md Yousof Ali, E.J. Kim, Y.M. Kim, H.A. Jung, Effects of C-glycosylation on anti-diabetic, anti-Alzheimer's disease and anti-inflammatory potential of apigenin, Food Chem. Toxicol. 64 (2014) 27–33, https://doi.org/10.1016/j.fct.2013.11.020.

[12] G.M. Cooper, R.E. Hausman. The Cell: A molecular Approach, Seventh edition.,, Sinauer Associates, Inc, Sunderland, Massachusetts U.S.A, 2016.

[13] S.R. Datta, A.M. Ranger, M.Z. Lin, J.F. Sturgill, Y.-C. Ma, C.W. Cowan, P. Dikkes, S. J. Korsmeyer, M.E. Greenberg, Survival factor-mediated BAD phosphorylation raises the mitochondrial threshold for apoptosis, Dev. Cell 3 (2002) 631–643, https://doi.org/10.1016/S1534-5807(02)00326-X.

[14] J. Dong, J. Qiu, J. Wang, H. Li, X. Dai, Y. Zhang, X. Wang, W. Tan, X. Niu, X. Deng, S. Zhao, Apigenin alleviates the symptoms of *Staphylococcus aureus* pneumonia by inhibiting the production of alpha-hemolysin, FEMS Microbiol Lett. 338 (2013) 124–131, https://doi.org/10.1111/1574-6968.12040.

[15] H. Hua-Xiang Xia, N.J. Talley, Apoptosis in gastric epithelium induced by helicobacter pylori infection: implications in gastric carcinogenesis, Am. J. Gastroenterol. 96 (2001) 16–26, https://doi.org/10.1111/j.1572-0241.2001.03447.x.

[16] M. Imran, T. Aslam Gondal, M. Atif, M. Shahbaz, T. Batool Qaisarani, M. Hanif Mughal, B. Salehi, M. Martorell, J. Sharifi-Rad, Apigenin as an anticancer agent, Phytother. Res. 34 (2020) 1812–1828, https://doi.org/10.1002/ptr.6647.

[17] D.H. Kim, N. Shin, G.H. Kim, G.A. Song, T.-Y. Jeon, D.-H. Kim, G.Y. Lauwers, D. Y. Park, Mucin expression in gastric cancer: reappraisal of its clinicopathologic and prognostic significance, Arch. Pathol. Lab. Med. 137 (2013) 1047–1053, https://doi.org/10.5858/arpa.2012-0193-OA.

[18] C.-H. Kuo, B.-C. Weng, C.-C. Wu, S.-F. Yang, D.-C. Wu, Y.-C. Wang, Apigenin has anti-atrophic gastritis and anti-gastric cancer progression effects in Helicobacter pylori -infected Mongolian gerbils, J. Ethnopharmacol. 151 (2014) 1031–1039, https://doi.org/10.1016/j.jep.2013.11.040.

[19] M.J. Leach, A.T. Page, Herbal medicine for insomnia: a systematic review and meta-analysis, Sleep. Med. Rev. 24 (2015) 1–12, https://doi.org/10.1016/j.smrv.2014.12.003.

[20] W.-J. Lee, W.-K. Chen, C.-J. Wang, W.-L. Lin, T.-H. Tseng, Apigenin inhibits HGF-promoted invasive growth and metastasis involving blocking PI3K/Akt pathway and β4 integrin function in MDA-MB-231 breast cancer cells, Toxicol. Appl. Pharmacol. 226 (2008) 178–191, https://doi.org/10.1016/j.taap.2007.09.013.

[21] R. Liu, T. Zhang, H. Yang, X. Lan, J. Ying, G. Du, The flavonoid apigenin protects brain neurovascular coupling against amyloid-β25-35-induced toxicity in mice, JAD 24 (2011) 85–100, https://doi.org/10.3233/JAD-2010-101593.

[22] Y. Liu, Y. Shi, R. Han, C. Liu, X. Qin, P. Li, R. Gu, Signaling pathways of oxidative stress response: the potential therapeutic targets in gastric cancer, Front Immunol. 14 (2023) 1139589, https://doi.org/10.3389/fimmu.2023.1139589.

[23] J. Machlowska, J. Baj, M. Sitarz, R. Maciejewski, R. Sitarz, Gastric cancer: epidemiology, risk factors, classification, genomic characteristics and treatment strategies, IJMS 21 (2020) 4012, https://doi.org/10.3390/ijms21114012.

[24] A. Marques-Ramos, R. Cervantes, Expression of mTOR in normal and pathological conditions, Mol. Cancer 22 (2023) 112, https://doi.org/10.1186/s12943-023-01820-z.

[25] G. Moghaddam, S.A. Ebrahimi, N. Rahbar-Roshandel, A. Foroumadi, Antiproliferative activity of flavonoids: influence of the sequential methoxylation state of the flavonoid structure: antiproliferative flavonoids from dracocephalum kotschyi, Phytother. Res. 26 (2012) 1023–1028, https://doi.org/10.1002/ptr.3678.

[26] S. Mushtaq, B.H. Abbasi, B. Uzair, R. Abbasi, Natural products as reservoirs of novel therapeutic agents, EXCLI J. 17 (2018) 420–451, https://doi.org/10.17179/excli2018-1174.

[27] S.F. Nabavi, H. Khan, G. D'onofrio, D. Šamec, S. Shirooie, A.R. Dehpour, S. Argüelles, S. Habtemariam, E. Sobarzo-Sanchez, Apigenin as neuroprotective agent: of mice and men, Pharmacol. Res. 128 (2018) 359–365, https://doi.org/10.1016/j.phrs.2017.10.008.

[28] C.H. Ooi, T. Ivanova, J. Wu, M. Lee, I.B. Tan, J. Tao, L. Ward, J.H. Koo, V. Gopalakrishnan, Y. Zhu, L.L. Cheng, J. Lee, S.Y. Rha, H.C. Chung, K. Ganesan, J. So, K.C. Soo, D. Lim, W.H. Chan, W.K. Wong, D. Bowtell, K.G. Yeoh, H. Grabsch, A. Boussioutas, P. Tan, Oncogenic pathway combinations predict clinical prognosis in gastric cancer, PLoS Genet 5 (2009) e1000676, https://doi.org/10.1371/journal.pgen.1000676.

[29] B. Oosterlinck, H. Ceuleers, W. Arras, J.G. De Man, K. Geboes, H. De Schepper, M. Peeters, S. Lebeer, J. Skieceviciene, G.L. Hold, J. Kupcinskas, A. Link, B.Y. De Winter, A. Smet, Mucin-microbiome signatures shape the tumor microenvironment in gastric cancer, Microbiome 11 (2023) 86, https://doi.org/10.1186/s40168-023-01534-w.

[30] M.J. Page, J.E. McKenzie, P.M. Bossuyt, I. Boutron, T.C. Hoffmann, C.D. Mulrow, L. Shamseer, J.M. Tetzlaff, E.A. Akl, S.E. Brennan, R. Chou, J. Glanville, J. M. Grimshaw, A. Hróbjartsson, M.M. Lalu, T. Li, E.W. Loder, E. Mayo-Wilson, S. McDonald, L.A. McGuinness, L.A. Stewart, J. Thomas, A.C. Tricco, V.A. Welch, P. Whiting, D. Moher, The PRISMA 2020 statement: an updated guideline for reporting systematic reviews, PLoS Med. 18 (2021) e1003583, https://doi.org/10.1371/journal.pmed.1003583.

[31] PDQ Adult Treatment Editorial Board, Gastric cancer treatment (PDQ®): health professional version. PDQ Cancer Information Summaries, National Cancer Institute (US), Bethesda (MD), 2002.

[32] A.H. Rahmani, M.A. Alsahli, A. Almatroudi, M.A. Almogbel, A.A. Khan, S. Anwar, S.A. Almatroodi, The potential role of apigenin in cancer prevention and treatment, Molecules 27 (2022) 6051, https://doi.org/10.3390/molecules27186051.

[33] V. Rajendran, M.V. Jain, In vitro tumorigenic assay: colony forming assay for cancer stem cells, in: G. Papaccio, V. Desiderio (Eds.), Cancer Stem Cells, Springer New York, New York, NY, 2018, pp. 89–95.

[34] A.M. Ranger, J. Zha, H. Harada, S.R. Datta, N.N. Danial, A.P. Gilmore, J.L. Kutok, M.M. Le Beau, M.E. Greenberg, S.J. Korsmeyer, *Bad* -deficient mice develop diffuse large B cell lymphoma, Proc. Natl. Acad. Sci. USA 100 (2003) 9324–9329, https://doi.org/10.1073/pnas.1533446100.

[35] R.R. Ruela-de-Sousa, G.M. Fuhler, N. Blom, C.V. Ferreira, H. Aoyama, M. P. Peppelenbosch, Cytotoxicity of apigenin on leukemia cell lines: implications for prevention and therapy, e19–e19, Cell Death Dis. 1 (2010), https://doi.org/10.1038/cddis.2009.18.

[36] B. Salehi, A. Venditti, M. Sharifi-Rad, D. Kręgiel, J. Sharifi-Rad, A. Durazzo, M. Lucarini, A. Santini, E. Souto, E. Novellino, H. Antolak, E. Azzini, W. Setzer, N. Martins, The therapeutic potential of apigenin, IJMS 20 (2019) 1305, https://doi.org/10.3390/ijms20061305.

[37] H.-S. Seo, M.A. Sikder, H.J. Lee, J. Ryu, C.J. Lee, Apigenin inhibits tumor necrosis factor-α-induced production and gene expression of mucin through regulating nuclear factor-kappa b signaling pathway in airway epithelial cells, Biomol. Ther. 22 (2014) 525–531, https://doi.org/10.4062/biomolther.2014.094.

[38] S. Shukla, S. Gupta, Apigenin-induced prostate cancer cell death is initiated by reactive oxygen species and p53 activation, Free Radic. Biol. Med. 44 (2008) 1833–1845, https://doi.org/10.1016/j.freeradbiomed.2008.02.007.

[39] S. Shukla, E. Shankar, P. Fu, G.T. MacLennan, S. Gupta, Suppression of NF-κB and NF-κB-regulated gene expression by apigenin through IκBα and IKK pathway in TRAMP mice, PLoS ONE 10 (2015) e0138710, https://doi.org/10.1371/journal.pone.0138710.

[40] R. Sitarz, M. Skierucha, J. Mielko, J. Offerhaus, R. Maciejewski, W. Polkowski, Gastric cancer: epidemiology, prevention, classification, and treatment, CMAR Volume 10 (2018) 239–248, https://doi.org/10.2147/CMAR.S149619.

[41] H. Slika, H. Mansour, N. Wehbe, S.A. Nasser, R. Iratni, G. Nasrallah, A. Shaito, T. Ghaddar, F. Kobeissy, A.H. Eid, Therapeutic potential of flavonoids in cancer: ROS-mediated mechanisms, Biomed. Pharmacother. 146 (2022) 112442, https://doi.org/10.1016/j.biopha.2021.112442.

[42] T. Su, C. Huang, C. Yang, T. Jiang, J. Su, M. Chen, S. Fatima, R. Gong, X. Hu, Z. Bian, Z. Liu, H.Y. Kwan, Apigenin inhibits STAT3/CD36 signaling axis and reduces visceral obesity, Pharmacol. Res. 152 (2020) 104586, https://doi.org/10.1016/j.phrs.2019.104586.

[43] T. Su, C. Huang, C. Yang, T. Jiang, J. Su, M. Chen, S. Fatima, R.-H. Gong, X. Hu, Z. Bian, Z. Liu, H.Y. Kwan, Corrigendum to "Apigenin inhibits STAT3/CD36 signaling axis and reduces visceral obesity" [Pharmacol. Res. (2020) 152 104586, Pharmacol. Res. 193 (2023) 106816, https://doi.org/10.1016/j.phrs.2023.106816.

[44] R.C. Taylor, S.P. Cullen, S.J. Martin, Apoptosis: controlled demolition at the cellular level, Nat. Rev. Mol. Cell Biol. 9 (2008) 231–241, https://doi.org/10.1038/nrm2312.

[45] E.G. Tsalkidou, A.K. Tsaroucha, E. Chatzaki, M. Lambropoulou, F. Papachristou, G. Trypsianis, M. Pitiakoudis, G. Vaos, C. Simopoulos, The effects of apigenin on the expression of fas/fasl apoptotic pathway in warm liver ischemia-reperfusion injury in rats, BioMed. Res. Int. 2014 (2014) 1–7, https://doi.org/10.1155/2014/157216.

[46] R. Van Dross, Y. Xue, A. Knudson, J.C. Pelling, The chemopreventive bioflavonoid apigenin modulates signal transduction pathways in keratinocyte and colon carcinoma cell lines, J. Nutr. 133 (2003) 3800S–3804S, https://doi.org/10.1093/jn/133.11.3800S.

[47] J. Wang, Y.-T. Liu, L. Xiao, L. Zhu, Q. Wang, T. Yan, Anti-inflammatory effects of apigenin in lipopolysaccharide-induced inflammatory in acute lung injury by suppressing COX-2 and NF-kB pathway, Inflammation 37 (2014) 2085–2090, https://doi.org/10.1007/s10753-014-9942-x.

[48] M. Wang, J. Firrman, L. Liu, K. Yam, A review on flavonoid apigenin: dietary intake, ADME, antimicrobial effects, and interactions with human gut microbiota, BioMed. Res. Int. 2019 (2019) 1–18, https://doi.org/10.1155/2019/7010467.
[49] Y.-C. Wang, K.-M. Huang, In vitro anti-inflammatory effect of apigenin in the Helicobacter pylori-infected gastric adenocarcinoma cells, Food Chem. Toxicol. 53 (2013) 376–383, https://doi.org/10.1016/j.fct.2012.12.018.
[50] K. Wu, L.-H. Yuan, W. Xia, Inhibitory effects of apigenin on the growth of gastric carcinoma SGC-7901 cells, World J. Gastroenterol. 11 (2005) 4461–4464, https://doi.org/10.3748/wjg.v11.i29.4461.
[51] F. Xia, X. Li, B. Wang, P. Gong, F. Xiao, M. Yang, L. Zhang, J. Song, L. Hu, M. Cheng, C. Sun, X. Feng, L. Lei, S. Ouyang, Z.-J. Liu, X. Li, J. Gu, W. Han, Combination Therapy of LysGH15 and apigenin as a new strategy for treating pneumonia caused by Staphylococcus aureus, Appl. Environ. Microbiol 82 (2016) 87–94, https://doi.org/10.1128/AEM.02581-15.
[52] X. Yan, M. Qi, P. Li, Y. Zhan, H. Shao, Apigenin in cancer therapy: anti-cancer effects and mechanisms of action, Cell Biosci. 7 (2017) 50, https://doi.org/10.1186/s13578-017-0179-x.
[53] J. Yang, C. Pi, G. Wang, Inhibition of PI3K/Akt/mTOR pathway by apigenin induces apoptosis and autophagy in hepatocellular carcinoma cells, Biomed. Pharmacother. 103 (2018) 699–707, https://doi.org/10.1016/j.biopha.2018.04.072.
[54] L. Yuan, W. Xia, X. Zhao, X. Zhang, L. Zhang, K. Wu, Inhibition of PKB/Akt activity involved in apigenin-induced apoptosis in human gastric carcinoma cells, Chin. Sci Bull 52 (2007) 2226–2232, https://doi.org/10.1007/s11434-007-0342-6.
[55] K. Zhang, W. Song, D. Li, X. Jin, Apigenin in the regulation of cholesterol metabolism and protection of blood vessels, Exp. Ther. Med. 13 (2017) 1719–1724, https://doi.org/10.3892/etm.2017.4165.
[56] G. Zhao, X. Han, W. Cheng, J. Ni, Y. Zhang, J. Lin, Z. Song, Apigenin inhibits proliferation and invasion, and induces apoptosis and cell cycle arrest in human melanoma cells, Oncol. Rep. 37 (2017) 2277–2285, https://doi.org/10.3892/or.2017.5450.
[57] L. Zhao, J.-L. Wang, R. Liu, X.-X. Li, J.-F. Li, L. Zhang, Neuroprotective, anti-amyloidogenic and neurotrophic effects of apigenin in an Alzheimer's disease mouse model, Molecules 18 (2013) 9949–9965, https://doi.org/10.3390/molecules18089949.