Phytochemicals in cancer and their effect on the PI3K/AKT-mediated cellular signalling

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ABSTRACT

Protein kinases belong to the largest family of enzymes controlling every aspect of cellular activity including gene expression, cell division, differentiation and metabolism. They are part of major intracellular signalling pathways. Hence, it is not surprising that they are involved in the development of major diseases such as cardiovascular disorders, diabetes, dementia and, most importantly, cancer when they undergo mutations, modifications and unbalanced expression.

This review will explore the possibility to draw a connection between the application of natural phytochemicals and the treatment of cancer. We have chosen to focus on the PI3K/AKT cellular signalling pathway which has been shown to be a major target by natural compounds in cell cultures and animal models.

1. Introduction

The consumption of plants for medicinal purposes, including cancer prevention and treatment, has a long history in various human civilizations. In this review, we would like to focus on phytochemicals that have been shown to influence cancer cells especially since 30–40% of cancers are known to be influenced by diet [1]. Hence, we will: (i) present a selection of phytochemicals present in our daily diet and discuss their potential to interfere with the PI3K/AKT signalling pathway and (ii) show data, where phytochemicals were used in vitro and in vivo alone or together with conventional chemotherapy to treat cancer (i.e., combination therapy) [2–5]. This is because natural compounds can intensify the action of the administered therapeutics, thus, causing cytotoxic effects on tumour cells. This strategy can reduce the patients’ burden following chemotherapy treatment alone by replacing part of it through a natural substance with a defined effect [6]. We will also (iii) discuss reports that phytochemicals such as curcumin and resveratrol have a proven preventive effect on our health [7,8] and consider the challenges behind their separation and purification. One of the challenges for the isolation of natural compounds is their low abundance in plants. Needless to say, that this also is a limiting factor when whole plants are used as a chemo-preventive treatment against cancer. The plant products are usually present as a complex with other part of it through a natural substance with a defined effect [6]. We will consider the challenges behind their separation and purification. One of the major classes, i.e., polyphenols and terpenoids (Table 1) [13]. Polyphenols are further divided into flavonoids and non-flavonoids. Virtually all of the compounds described in Table 1 are found in most of the food we consume, indicating that they are an important and vital contribution to our health [13–16]. Needless to say, that it does not come as a surprise that these compounds exhibit antioxidant properties that are important for the prevention and treatment of chronic diseases including cancer [6,14,17–19].

1.2. Polyphenols and terpenoids

Polyphenols comprise flavonoids and non-flavonoids [13]. These two groups are further divided into various subclasses. We show representative compounds for each subclass (Table 1). Apigenin, luteolin, baicalein and chrysin belong to the subclass of flavones. They are contained in citrus fruits, onions, cucumbers and strawberries, only to name a few of them present in food which we find in our daily recipes. Quercetin, kaempferol, fisetin belong to the family of flavans and are present in citrus fruits, olive oil, tea, red wine, onions, cucumbers, strawberries and many other plants. Naringenin, hesperidin, hesperetin belong to the flavonones, also found in citrus fruits, tomatoes and aromatic plants such as mint. More peculiar is the distribution of the

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Curcuma longa in the dried leaves of green and white tea. Anthocyanins, such as delphinidin and cyanidin are found in cranberries, concord grapes, pomegranate, etc. Ellagic acid is a member of the hydrobenzoic acids, while gallic acid, Ellagic Acid (fruits and vegetable, e.g., raspberries, strawberries, walnuts, pecans etc.) are present in natural sources like gallnuts, sumac, etc. The basic building blocks of polyphenols are benzoic acids and hydroxycinnamic acids. These acids can be found in various plants, vegetables, and fruits. A total of 13 protein kinases (PKs) were predominantly described in the reports we have investigated, yet phosphoinositide 3-kinase (PI3K), which comprises a family of lipid kinases [20], and AKT were described to be involved in the treatment of cancer cells with phytochemicals in the majority of the analysed reports. The PI3K/AKT/mTOR pathway regulates important cellular processes like cell growth, proliferation, apoptosis etc. [21]. Aberrant activation of this pathway promotes survival and proliferation of malignant cells. PI3K is one of the most frequently mutated protein kinase reported in tumour cells and it is a logic target for inhibition in combination with conventional chemotherapy and agents targeting other oncogenic proteins for the treatment of cancer [21]. Given the key role of PI3K/AKT pathway in cancer and other diseases, we have focused our review on phytochemicals interfering with this signalling cascade (Fig. 1). In a PubMed search between 2003 and 2021 using the search term: (PI3K, AKT signalling) AND (cancer) AND (compound name), 123 citations were found, for instance, for curcumin and 93 for resveratrol, respectively. Hence, the number of publications which appeared in the search, was relatively modest and yet, the PI3K/AKT pathway was affected in most of the cases, where natural compounds were used to treat tumours and cancer cells. A search taking into consideration other signalling cascades e.g., MAPK, although longer known than PI3K/AKT, showed a lower number of citations. This makes the importance and understanding of other protein kinases and signalling pathways in tumours and cancer cells more complex. It raises the question of relevance: i.e., is it more important to inhibit the PI3K/AKT cascade or other pathways such as the MAPK pathway? Is it an advantage to know certain cellular targets instead of inhibiting cell proliferation in general by using conventional chemotherapy based, for instance, on cisplatin, 5-FU etc.? More research is definitely required to answer this question.

### 3. Selection of phytochemicals and their involvement in PI3K/AKT signalling and therapeutic use

Next, we made a correlation between the selected phytochemicals and intracellular signalling pathways controlling cell proliferation and/or cell death (Table 2).

<table>
<thead>
<tr>
<th>Flavonoids</th>
<th>Subclass</th>
<th>Compound</th>
<th>Natural source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavones</td>
<td>Apigenin, Luteolin, Baicalin, Chrysin</td>
<td>Citrus fruits, onions, cucumbers, strawberries</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Quercetin, Fisetin</td>
<td>Citrus fruits, olive oil, tea, red wine, onions, cucumbers, strawberries</td>
<td></td>
</tr>
<tr>
<td>Flavanones</td>
<td>Naringenin, Hesperidin, Hesperetin</td>
<td>Tomatoes, citrus, aromatic plants (e.g., mint)</td>
<td></td>
</tr>
<tr>
<td>Flavanols</td>
<td>Stilbene, Silmarin, Taxifolin</td>
<td>Milk thistle (Silybum marianum), Taxifolin (Siberian larch, Larix sibirica)</td>
<td></td>
</tr>
<tr>
<td>Isoflavones</td>
<td>Genistein, Daidzin, Pomiferin</td>
<td>Lupine, fava beans, soybeans</td>
<td></td>
</tr>
<tr>
<td>Tannins</td>
<td>Flavan-3-ols/ Catechins</td>
<td>Dried leaves of green tea and white tea</td>
<td></td>
</tr>
<tr>
<td>Anthocyanins</td>
<td>Cyanidin, Delphinidin</td>
<td>Cranberries, concord grapes, pomegranates, bilberries</td>
<td></td>
</tr>
<tr>
<td>Lignans</td>
<td>Honokiol (Neolignan bisphenol)</td>
<td>Bark, seed cones and leaves of trees belonging to the genus Magnolia</td>
<td></td>
</tr>
<tr>
<td>Coumestans</td>
<td>Wedelolactone, Coumestrol</td>
<td>Plants belonging to the Asteraceae family</td>
<td></td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Curcumin, Boldine, Harmin</td>
<td>Indian spice Turmeric, Curcuma longa L. (a member of the ginger family)</td>
<td></td>
</tr>
<tr>
<td>Stilbenes, Tannins</td>
<td>Tetrahydroxystilbene</td>
<td>Polygonum multiflorum Thunb., a traditional Chinese medicinal herb</td>
<td></td>
</tr>
<tr>
<td></td>
<td>glucoside, ellagitannins</td>
<td>Resveratrol: grapes, cinnamon oil</td>
<td></td>
</tr>
<tr>
<td>Terpenoids</td>
<td>3,4',5-trihydroxystilbene (Resveratrol)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 1. Classification of major phytochemicals.

<table>
<thead>
<tr>
<th>Polyphenols</th>
<th>Subclass</th>
<th>Compound</th>
<th>Natural source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoids</td>
<td>Monoterpenoids</td>
<td>Cantharidin, Limonene</td>
<td>Citrus fruit peels, male blister beetle</td>
</tr>
<tr>
<td></td>
<td>Sesquiterpenoids</td>
<td>Parthenolide, Artemisin</td>
<td>Feverfew (Tanacetum parthenium)</td>
</tr>
<tr>
<td></td>
<td>Diterpenoids</td>
<td>Oridonin</td>
<td>Rubosia rubescens</td>
</tr>
<tr>
<td></td>
<td>Triterpenoids</td>
<td>Celastrol</td>
<td>Root extracts Tripterygium wilfordii (Thunder god vine)</td>
</tr>
<tr>
<td></td>
<td>Triterpenoids</td>
<td>Carotenoids, Lycopenes</td>
<td>Various plants, vegetables, and fruits</td>
</tr>
</tbody>
</table>

flavonoids silibinin, silimarum, and taxifolin. These compounds are found in milk thistle, the siberian larch and therefore not available through the common daily diet. Genistein, daidzin and pomiferin belong to the subclass of isoflavones and are foremost found in lupine, fava and soybeans. Epigallocatechin-3-gallate (EGCG) is found in the dried leaves of green and white tea. Anthocyanins, such as delphinidin and cyanidin are found in cranberries, concord grapes, pomegranates, etc. The second class of phytochemicals are the non-flavonoids which comprise five subclasses, i.e., phenolic acids (hydroxybenzoic acids and hydroxycinnamic acids), lignans, coumestans, alkaloids, stilbenes and tannins [13]. Gallic and ellagic acid are members of the hydrobenzoic acids and are found in gall nuts, witch hazel and tea leaves. Honokiol, is a natural bisphenol neolignan, isolated from the bark, seed cones and leaves of trees belonging to the genus Magnolia. The alkaloids are a fairly large family, and we would like to mention curcumin, boldine, and harmine. They are derived from the popular indian spice turmeric, Curcuma longa, a member of the ginger family. Last not least, the subclass of stilbenes and tannins should be mentioned. Compounds which belong to this subclass of polyphenols are tetrahydroxystilbene glucosides, ellagitannins and 3,4',5-trihydroxystilbene (resveratrol).

Another class of phytochemicals are the terpenoids which comprise compounds such as limonene and cantharidin, found in the oil from citrus fruit peels and the male blister beetle. Partenholide and artemisin (sesquiterpenoids) are found in the plant feverfew. Oridonin and celastrol belong to the subclass of diterpenoids and triterpenoids, respectively. They are present in Rubosia rubescens and the root extracts from Tripterygium wilfordii. The final subclass are the tetraterpenoids to which the carotenoids belong. These are pigments that produce the bright yellow, red and orange colours in plants, vegetables and fruits. They act as antioxidants.

### 2. Protein kinases and phytochemicals

We have selected 19 phytochemicals from the compound listing in Table 1. A total of 13 protein kinases (PKs) were predominantly described in the reports we have investigated, yet phosphoinositide 3-kinase (PI3K), which comprises a family of lipid kinases [20], and AKT were described to be involved in the treatment of cancer cells with phytochemicals in the majority of the analysed reports. The PI3K/AKT/mTOR pathway regulates important cellular processes like cell growth, proliferation, apoptosis etc. [21]. Aberrant activation of this pathway promotes survival and proliferation of malignant cells. PI3K is one of the most frequently mutated protein kinase reported in tumour cells and it is a logic target for inhibition in combination with conventional chemotherapy and agents targeting other oncogenic proteins for the treatment of cancer [21]. Given the key role of PI3K/AKT pathway in cancer and other diseases, we have focused our review on phytochemicals interfering with this signalling cascade (Fig. 1).
A

Cell Survival
Proliferation
Metabolism

Cell Growth
Protein Synthesis

RTK

PTEN

PIP2

IRS

GRB2

Ras

Rac

MLK

MEKK1/4

MEKK3/6

SEK

JNK

p38 MAPK

CK2

Rheb

AMPK

mTOR

Rictor

mTORC1

mTORC2

TSC1

TSC2

AKT

ERK1/2

Transcription
Cell proliferation
Growth
Cell survival
Differentiation

B

Co-receptor
Frizzled receptor

CK1

Axin

Dishevelled

GSK-3β

CK2

APC

Axin

GSK-3β

CK1

b-Catenin

CK2

Apoptosis

Nucleus

b-Catenin

Transactivation
of target genes

C

L

Receptor

JAK

JAK

JAK

JAK

STAT

L

L

STAT

STAT

Transactivation
of target genes

(caption on next page)
particles to induce autophagy to inhibit the PI3K/AKT/mTOR cascade in treatment with focus on the PI3K/AKT pathway is provided by Ham of the many effects that have been ascribed to this polyphenol. A nice exhibit antioxidant and anti-inflammatory activities just to name a few.

Overview of major signalling pathways mentioned in the review. (A) The PI3K/AKT signalling cascade is activated upon binding of growth factors to the appropriate receptor tyrosine kinase (RTK). Phosphoinositide 3-kinase (PI3K) becomes activated by means of certain adaptor proteins (IRS) bound to the RTK and phosphorylates specific membrane lipids resulting in the activation of downstream protein kinases such as PDK1 and AKT. AKT is a positive regulator of mTOR in complex with Raptor also known as mTORC1 complex. Active mTOR phosphorylates, among others, p70S6K which results in the stimulation of protein synthesis and cell growth. mTOR in complex with Rictor (i.e., mTORC2 complex) and CK2, respectively, can phosphorylate AKT promoting its activity. Persistent activation of AKT confers cell survival, enhances cell proliferation and cellular metabolic activities. A large variety of extracellular signals may also activate RTKs and be transduced through the cytoplasm by a cascade of protein kinases ultimately stimulating the activity of ERK1/2, JNK and p38MAPK, respectively. These downstream targets control a plethora of cellular responses as indicated in the figure. (B) Simplified overview of the canonical Wnt signalling cascade. Wnt proteins bind to the N-terminal extra-cellular domain of the Frizzled receptor and a co-receptor. After binding of Wnt to the receptor complex, the signal is transduced to cytoplasmic proteins such as Dishevelled, GSK-3β, Axin, Adenomatous polyposis coli (APC) and CK1. Binding of Wnt to the Frizzled receptor triggers a series of events that disrupts the APC/Axin/GSK-3β complex that is required for the targeted degradation of β-catenin. In the nucleus, β-catenin regulates gene transcription by functioning as a transcriptional co-activator. Protein kinase CK2 and a number of other protein kinases can phosphorylate Dishevelled and regulate its subcellular localization and ability to interact with other proteins. (C) The mammalian JAK/STAT pathway is activated by the initial binding of a ligand (L) to a transmembrane receptor. Ligand binding causes a conformational change of the receptor and phosphorylation in trans of JAK. In turn, activated JAK phosphorylates the receptor, and an inactive STAT dimer is recruited to the receptor at those phospho-sites where it is phosphorylated by active JAK. The phosphorylated STAT dimers translocate into the nucleus, bind specific DNA sequences on target genes and trigger a specific cellular response.

Concerning the involvement of cantharadin in cancer came from [25]. These authors reported that cantharadin suppresses cell growth and migration in non-small lung cancer cells. Cantharadin interferes with cell growth and migration by inhibiting the PI3K/AKT/mTOR signalling in these cancer cells and promotes autophagy and apoptosis.

Celastrol is a triterpene compound derived from TCM. It is isolated from root extracts of *Tripterygium wilfordii* and has been reported to possess potential anti-tumour activity. Bufu et al., [26] showed that celastrol inhibits colorectal cancer cell proliferation and migration through suppression of matrix metalloproteinases 3 (MMP3) and 7 (MMP7) by the PI3K/AKT signalling pathway. Celastrol inhibits colon cancer cell proliferation by down-regulating miR-21 and PI3K/AKT/GSK-3β pathway [27]. Anti-tumour activity of celastrol by inhibition of proliferation, invasion and migration in cholangiocarcinoma via the PTEN/PI3K/AKT pathway was also described by Zhu and Wei [28].

Chrysos has been shown to suppress PI3K and influence the expression of AKT, p70S6K, S6 and p90RSK proteins. Ryu et al. [29] showed that chrysos induces death of prostate cancer cells by inducing reactive oxygen species (ROS) and endoplasmic reticulum (ER) stress through suppression of the PI3K/AKT signalling. Very interesting is the article by Yang et al. [30] which describes the inhibition of the metastatic potential of human triple-negative breast cancer cells by modulation of epithelial-to-mesenchymal transition (EMT) and PI3K/AKT signalling. Chrysos has also been shown to enhance the sensitivity of tumour cells to doxorubicin by suppressing the PI3K/AKT cascade [31].

Curcumin, a component of a spice native to India, has been shown to exhibit antioxidant and anti-inflammatory activities just to name a few of the many effects that have been ascribed to this polyphenol. A nice overview on the versatile role of curcumin in cancer prevention and treatment with focus on the PI3K/AKT pathway is provided by Hamzehzadeh et al., [32]. Maiz et al., [33] have used solid lipid curcumin particles to induce autophagy to inhibit the PI3K/AKT/mTOR cascade in cultured glioblastoma cells. Wang et al., [34] showed that curcumin inhibits migration and invasion of non-small cell lung cancer cells through up-regulation of miR-206 and suppression of PI3K/AKT/mTOR signalling axis. Finally, curcumin has also been shown to down-regulate the PI3K/AKT/mTOR pathway and inhibit growth and progression of head and neck cancer cells [35].

Cyanidin belongs to the anthocyanins, a subclass of flavonoids found in cranberries, concord grapes, pomegranates and bilberries just to name a few fruits. Anthocyanins are the pigments that give red, purple, and blue plants their rich colouring. In addition to acting as antioxidants and fighting free radicals, anthocyanins may offer anti-inflammatory, anti-viral, and anti-cancer benefits. Ya et al. [36] showed that cyanidin induces megakaryocyte apoptosis via PI3K/AKT inhibition of NF-kB signalling. Moreover, Li et al., [37] showed that cyanidin inhibits EMT induced by oxaliplatin via targeting the PDK1-PI3K/AKT signalling cascade.

Epigallocatechin gallate (EGCG), also known as epigallocatechin-3-gallate, is the ester of epigallocatechin and gallic acid, and is a type of catechin. It is predominantly found in dried leaves of green and white tea. Moradzadeh et al., [38] showed that EGCG promotes apoptosis in human breast cancer cells through down-regulation of PI3K/AKT and telomerase. EGCG can also down-regulate doxorubicin-induced over-expression of P-glycoprotein through the PI3K/AKT pathway [39]. A third example showing the involvement of EGCG on PI3K/AKT signalling derives from Chakrabarty et al., [40]. The authors showed that theaflavin and EGCG induce apoptosis through the inhibition of the PI3K/AKT cascade.

Khan et al., [41] could show that treatment of PI3KCA-mutant cells with fisetin and 5-FU reduces the expression of PI3K-mediated phosphorylation of AKT and mTOR and increased the phosphorylation of AMPK-alpha. Tumour incidence was markedly lower in fisetin-treated mice that also expressed constitutively active PI3K as compared to control animals, suggesting that fisetin could be used as a complementary agent when applied together with 5-FU treatment. Sun et al., [12] reported on the anti-cancer effects of fisetin on breast cancer cell lines via the PI3K/AKT/mTOR pathway. However, disappointingly, in vivo studies in tumour-bearing mice showed no such effect because of the low solubility and low bioavailability of this compound [41].

Gallic acid (GA), a non-flavonoid compound, belongs to the group of phenolic acids. It is found in gallnuts, sumac, witch hazel oak and bark (Table 1). Su et al. [42] described inhibition of melanogenesis by GA, where among other molecules also the PI3K/AKT signalling is involved. Zeng et al. [43] showed that GA inhibits bladder cancer cell progression where among other molecules also the PI3K/AKT signalling is involved. Moreover, Li et al., [37] showed that cyanidin inhibits EMT induced by oxaliplatin via targeting the PDK1-PI3K/AKT signalling cascade.

Honokiol has been shown to exhibit long-term pharmacological effects, e.g., being anti-bacterial, anti-spasmodic, anti-anxiety and anti-viral. It is clinically used in the treatment of acute enteritis and chronic gastritis. Li et al. [47] have investigated a possible role of honokiol with respect to induction of autophagy and/or apoptosis in osteosarcoma cells. The authors showed that honokiol could inhibit cell proliferation and enhance the apoptotic rate of cancer cells and these effects were linked to the PI3K/AKT/mTOR signalling pathway. Honokiol has also been shown to suppress proliferation and to induce apoptosis via...
Table 2
Selection of phytochemicals affecting the PI3K/AKT signalling in cancer and corresponding therapeutic use.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Therapeutic use</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apigenin</td>
<td>Hepatocarcinoma cells</td>
<td>[22,23]</td>
</tr>
<tr>
<td></td>
<td>Different human cancers and cell lines</td>
<td></td>
</tr>
<tr>
<td>Cantharidin</td>
<td>Gastric cancer cells</td>
<td>[24,25]</td>
</tr>
<tr>
<td>Celastrol</td>
<td>Non-small-cell lung carcinoma (NSCLG)</td>
<td>[26-28]</td>
</tr>
<tr>
<td>Chrysin</td>
<td>Prostate cancer cells</td>
<td>[29-31]</td>
</tr>
<tr>
<td>Curcumin</td>
<td>Various tumour cell lines and tumour</td>
<td>[32-35]</td>
</tr>
<tr>
<td>Cyanidin</td>
<td>Pro-apoptotic in human platelets</td>
<td>[36,37]</td>
</tr>
<tr>
<td>Epigallocatechin-3-gallate</td>
<td>Down-regulation of P-glycoprotein in HepG2 cells</td>
<td>[38-40]</td>
</tr>
<tr>
<td>Fisetin</td>
<td>Anti-proliferation in breast cancer cells</td>
<td>[12,41]</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>Inhibition of melanoma cell growth</td>
<td>[42,43]</td>
</tr>
<tr>
<td>Genistein</td>
<td>Inhibition of cell migration in ovarian cancer</td>
<td>[44-46]</td>
</tr>
<tr>
<td>Honokiol</td>
<td>Induction of autophagy/apoptosis in osteosarcoma cells</td>
<td>[47-49]</td>
</tr>
<tr>
<td></td>
<td>Suppression of proliferation in osteosarcoma cells</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Autophagic cell death in malignant glioma cells</td>
<td></td>
</tr>
<tr>
<td>Luteolin</td>
<td>Apoptosis in Tamoxifene-resistant breast cancer cells</td>
<td>[50-52]</td>
</tr>
<tr>
<td></td>
<td>Reduction of migration in glioblastoma cells</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Induction of apoptosis in choriocarcinoma cells</td>
<td></td>
</tr>
<tr>
<td>Lycopene</td>
<td>Inhibition of EMT in oral cancer</td>
<td>[53,54]</td>
</tr>
<tr>
<td>Naringenin</td>
<td>Inhibition of prostate cancer cell growth</td>
<td>[55-57]</td>
</tr>
<tr>
<td></td>
<td>Migration of embryonic trophoblast cells</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Apoptotic cell death in prostate cancer cells</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Inhibition of thyroid cancer cells</td>
<td></td>
</tr>
<tr>
<td>Oridonin</td>
<td>Inhibition of oral cancer cell growth</td>
<td>[58-60]</td>
</tr>
<tr>
<td></td>
<td>Induction of apoptosis in oesophageal cancer cells</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Induction of apoptosis in prostate cancer cells</td>
<td></td>
</tr>
<tr>
<td>Parthenolide</td>
<td>Inhibition of lung cancer cell growth</td>
<td>[61-63]</td>
</tr>
<tr>
<td></td>
<td>Inhibition of proliferation in thyroid carcinoma cells</td>
<td></td>
</tr>
<tr>
<td>Resveratrol</td>
<td>Inhibition of migration of hepatocellular carcinoma cells</td>
<td>[64-67]</td>
</tr>
<tr>
<td></td>
<td>Induction of apoptosis in cervical cancer</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Induction of proliferation in prostate cancer cells</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Induction of apoptosis in small cell lung cancer</td>
<td></td>
</tr>
<tr>
<td>Silibinin</td>
<td>Upregulation of BMP7 in colon carcinoma</td>
<td>[58-70]</td>
</tr>
<tr>
<td></td>
<td>Inhibition of cell invasion in lung carcinoma cells</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Induction of apoptosis in multiple myeloma cells</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Inhibition of cell proliferation in bladder cancer</td>
<td></td>
</tr>
</tbody>
</table>

regulation of miR-21/PTEN/PI3K/AKT signalling cascade in human osteosarcoma cells [48]. Moreover, Lin et al. [49] showed that honokiol can induce autophagic cell death in malignant glioma through ROS-mediated regulation of the p53/PI3K/AKT/mTOR signalling pathway.

Luteolin is a flavone found in citrus fruits, onions, cucumber and strawberries, just to mention a few plants. Luteolin is a common dietary flavonoid shown to exert anti-cancer effects in multiple cancer models. Wu et al. [50] explored the effect of luteolin on tamoxifen-resistant breast cancer cells. The compound caused apoptosis through the suppression of the PI3K/AKT/mTOR cascade. Wang et al. [51] showed that luteolin reduces migration of human glioblastoma cells by negatively affecting the p-IGF-IR/PI3K/AKT/mTOR signalling axis while Lim et al. [52] demonstrated inhibition of proliferation and induction of apoptosis of human placental choriocarcinoma cell by blocking the PI3K/AKT pathway.

Lycopene exhibits anti-cancer activity but also decreases the occurrence of chronic diseases including type 2 diabetes, osteoporosis, and cardiovascular disorders. Since lycopene has 11 conjugated double bonds, it is considered as one of the most potent antioxidants among known carotenoids. Wang et al. [53] showed that lycopene inhibits EMT and promoted apoptosis in oral cancer cell lines via the PI3K/AKT/mTOR signalling cascade. Another report on the effect of lycopene on the PI3K/AKT signalling pathway comes from Chen et al. [54]. These authors showed that lycopene has a positive effect on prostate cancer. The advantage of the application of lycopene for its anti-prostate cancer activity is that lycopene can reach much higher concentration in prostate tissue than other tissues. Based on evidence, they indicate that the PI3K/AKT pathway could be one of the major mechanisms for anti-cancer activity targeted by lycopene.

Naringenin is a flavanone, found in tomatoes, citrus fruit and aromatic plants like mint. Naringenin-induced apoptotic cell death in prostate cancer cells is mediated by the PI3K/AKT and MAPK signalling pathways [55]. Interestingly, the authors could show that naringenin enhances the efficiency of paclitaxel to suppress progression of prostate cancer cells. Lim et al. [56] demonstrated apoptotic cell death in prostate cancer cells via the PI3K/AKT and MAPK signalling cascade, induced by naringenin. Their results, using PC3 and LNCaP cells, revealed that naringenin inhibited proliferation and migration, while inducing apoptosis and ROS production in those cells. Another report using naringenin is provided by Zhou et al. [57]. These authors showed that this compound inhibits thyroid cancer cell proliferation and induces cell apoptosis through repression of the PI3K/AKT pathway.

Oridonin is a bioactive diterpenoid purified from Rabdosia rubescens. It has been shown to possess anticancer effects in several malignancies. Yang et al. [58] investigated this compound in oral squamous cell carcinoma. They could show that oridonin inhibits oral cancer growth by inhibiting the PI3K/AKT signalling pathway.

Jiang et al. [59] demonstrated that oridonin induces mitochondria-dependent apoptosis by inhibiting the PI3K/AKT/mTOR and RAS/RAF pathways in oesophageal cancer cells. Lu et al. [60] investigated the effect of oridonin on prostate cancer cells. Their results show that oridonin not only inhibited cell proliferation and clonal formation but also induced G2/M cell cycle arrest and apoptosis via the PI3K/AKT signalling cascade.

Parthenolide is a sesquiterpenoid found in the plant feverfew (Tanacetum parthenium). The highest concentrations are found in flowers and fruits (Table 1). It has been reported to inhibit human lung cancer cell growth by modulating the IGF-IR/PI3K/AKT signalling pathway [61]. Moreover, Li et al. [62] demonstrated that parthenolide inhibits proliferation of papillary thyroid carcinoma cell in vitro and in mouse tumour xenografts by down-regulating the mTOR/PI3K/AKT signalling pathway. Jayamohan et al. [63] showed that parthenolide also targets the PI3K/AKT pathway in cervical cancer.

Resveratrol is a stilbene present in many plant species, e.g., grapes, peanuts and berries. It also is a phytoalexin, hence, it is produced in
plants in response to mechanical injury, fungal infection etc. It provides a number of anti-aging health benefits including improved metabolism, cardio protection and cancer prevention. However, there are also limitations, i.e., its poor bioavailability when taken orally. Chai et al. [64] examined the effects of resveratrol on hepatocellular cancer (HCC). The authors showed that resveratrol inhibited the viability and proliferation of cancer cells through SIRT1-mediated post-translational modification of PI3K/AKT signalling. Ye et al. [65] who investigated the effect of resveratrol in prostate cancer cells, showed that this compound inhibits proliferation and promotes apoptosis via the PI3K/AKT signalling pathway. Similar results were reported by Li et al. [66]. These authors showed that resveratrol inhibits viability and induces apoptosis in small-cell lung cancer via the PI3K/AKT/ε-MYC axis but also in colon cancer the compound was found to inactivate the PI3K/AKT signalling cascade [67].

Silibinin is a flavan-3-ol extracted from the milk thistle. It has anti-oxidant and anti-inflammatory activities. Silibinin inhibits cell invasion through inactivation of both PI3K/AKT and MAPK signalling [68]. Notably, this compound has also been shown to suppress cell proliferation and induce apoptosis of multiple myeloma cells via the PI3K/AKT/mTOR signalling pathway by Feng et al. [69]. Imai-Sumida et al. [70] demonstrated that silibinin can suppress bladder cancer through down-regulation of actin cytoskeleton and the PI3K/AKT signalling axis.

4. Combination of established chemotherapy with complementing natural compounds

An evolving approach is the combination of conventional chemotherapy together with natural compounds in patients. With respect to Table 2, oridonin and silibinin have been shown to enhance conventional chemotherapy. In the case of oridonin, it was demonstrated that it enhances 5-FU toxicity by induction of necroptotic death [71]. In the case of silibinin, it was sorafenib-mediated effect that was enhanced [72]. In studies with different cancer cell lines or cancer stem cells, the combination of fisetin and 5-FU [41], gemcitabine and harmine [73], paclitaxel and naringenin [56], respectively, improved the effectiveness of the conventional chemotherapeutic treatment. There are many other reports describing the combination of phytochemical and established chemotherapy as adjuvants for cancer therapy. We provide here a short listing of relevant articles [74–88]. Several studies have shown that dietary phytochemicals indeed can have an effect on tumour growth in vivo, e.g., lycopene reduced tumour size and plasma prostate-specific antigen levels in prostate cancer patients [89]. In the case of patients with advanced pancreatic cancer, significant tumour regression was observed after curcumin treatment [90]. Other reports showed the effect of curcumin both alone and in combination with chemotherapy [91]. Parthenolide augments the chemosensitivity of non-small-cell lung cancer to cisplatin through the PI3K/AKT signalling pathway [92].

5. Phytochemicals and protein kinase-mediated signalling in different disorders

5.1. Natural compounds have multifunctional properties supporting their usage in many different disorders

Table 2 shows a list of natural compounds, their effect on the PI3K/AKT signalling axis and some examples for their therapeutic use. All compounds are representatives of the various polyphenols which appear in Table 1 and are all commercially available. Most of them are described as having multiple effects on health. Although we focused on the effect of these compounds on cellular signalling with respect to cancer, almost all of the compounds are reported to also affect a number of other diseases. This is, for instance, the case of curcumin. Apart from melanoma, pancreatic and colon cancer, curcumin exhibits a big promise as a therapeutic agent for a number of other conditions. It is currently employed in human trials for the treatment of psoriasis, Alzheimer disease, diabetic nephropathy and various other pathological conditions (for a review see [93]). An expanding body of preclinical evidence suggests that EGCG, the major catechin found in green tea (Camellia sinensis), has also the potential to impact a variety of human illnesses. Apparently, EGCG functions as a powerful antioxidant, preventing oxidative damage in healthy cells, but also as an anti-angiogenic and anti-tumour agent and as a modulator of tumour cell response to chemotherapy. Much of the cancer chemo-preventive properties of green tea are mediated by EGCG that induces apoptosis and promotes cell growth arrest by altering the expression of cell cycle regulatory proteins, activating killer caspases, and suppressing oncogenic transcription factors and pluripotency maintain factors. In vitro studies have demonstrated that EGCG blocks carcinogenesis by affecting a wide array of signal transduction pathways, foremost the PI3K/AKT cascade but also Notch, JAK/STAT, MAPK, and Wnt (Fig. 1) have been reported. EGCG stimulates telomere fragmentation through inhibiting telomerase activity [94]. Its cancer preventive properties and mechanism of action at multiple levels result in the regulation of cancer cells growth and survival, angiogenesis and metastasis making it an interesting compound for medical use. Hence, EGCG could be useful either alone or in combination with conventional therapeutics for the prevention of tumour incidence and progression [94]. Finally, EGCG also has been shown to have a positive effect on chronic inflammatory diseases which increasingly affect millions of people globally [95].

Genistein has several molecular functions, it e.g., is an agonist of G-protein – coupled oestrogen receptor, activates PPARs, inhibits tyrosine kinases, is anti-helminthic, is involved in modulating vascular inflammation, a major event in the pathogenesis of atherosclerosis, and finally, is involved in cancer, especially cancer of the prostate, cervix, brain, breast and colon [96].

There are many reports on therapeutic applications of honokiol in cancer. These have been based mostly on observations using tissue cultures of human lung squamous carcinoma and glioblastoma cell culture cell lines (Lee et al., [97]) but also in many other cancers (Banik et al., [98]). Apart from that, another area where honokiol is used is neuronal diseases, especially mental disorders including depression. Honokiol has also been used to treat asthma, anxiety, stomach disorders and inflammation [99].

Many researchers reported that luteolin possesses antioxidant, anti-cancer, anti-inflammatory and neuroprotective effects [95,100,101]. Quercetin has been shown to inhibit prostate cancer by attenuating cell survival and inhibiting anti-apoptotic pathways [102]. Moreover, it has been shown to have potential for the prevention of bone destruction in rheumatoid arthritis. In another report, it was described the utilization of liposomal quercetin and gallic acid in localized treatment of vaginal Candida infections [103].

Apart from the role on cancer, compelling evidence has linked resveratrol with various diseases and organs, e.g. ophthalmic diseases [104], metabolic health and obesity [105], inflammatory bowel disease [106], and the vascular system [107].

As with resveratrol and many other natural compounds, silibinin has been used to treat diseases other than cancer. There is a report on the role of silibinin in the management of Diabetes mellitus and its complications [108]. Silibinin has also been shown to be a potential therapeutic tool in rheumatoid arthritis [109], Frederico et al., [110] reported on silibinin and chronic liver disease. These are only three examples of many, and many totally different diseases have been associated in one way or another with the ability of silibinin to interfere with the illness. It is important to note that the vast majority of data published on natural compounds have been produced using established cell lines, either from animal or human origin, mostly derived from various tumours. Alternatively, some experiments were performed in genetically modified mice which allowed the growth of hetero-transplanted human tumour cells. Only a very limited number of experiments involving the application of natural compounds were, however, performed in patients.
Taken everything into consideration we come to the conclusion that most of the selected natural compounds tackle a plethora of diseases. None of them with high specificity and none of them successfully curing critical illnesses, e.g., cancer. The reason for this lack of efficiency is obvious: all-natural compounds isolated from plants did not develop during evolution to target specific signalling pathways and proteins etc. They developed to protect the plant against outside invaders, e.g., viruses, bacteria, fungi, insect, animals etc. Naively, one could assume that what works for plants also should work for human beings, similar as in the case for antibiotics which are produced in fungi to protect against bacteria. The reason why antibiotics also work in humans is that they do not have to interfere with the human metabolism; by contrast, they target the bacteria directly as they do with soil fungi.

6. Obesity and cancer: phytochemical-based strategies to overcome a lethal combination

In 2007, the World Cancer Research Fund declared that obesity increases the incidence of a number of cancers like breast, endometrial, colorectal, kidney, oesophageal and probably other types as well [111].

Why does obesity cause enhanced incidence and mortality of several cancers? Obesity is a condition which contributes to the development of hyperinsulinemia and insulin resistance. Persistent high levels of insulin are associated to production of insulin-like growth factor (IGF-1) and can lead to increased release of steroid hormones like oestrogens, promoting cell proliferation and inhibiting apoptosis [112]. The adipose tissue is also an important source of adipokines (e.g., leptin, adiponectin, Interleukin-6 and TNF-α) that are soluble molecules linked to inflammation. As adipose tissue expands, the level of these adipokines increases leading to chronic inflammation which has been implicated in the initiation and progression of cancer [113,114].

A number of signalling pathways play a prominent role in facilitating tumour growth caused by excess visceral fat (Fig. 1). Here below, we discuss key molecular mechanisms linking the two conditions. The PI3K/AKT signalling pathway is required for normal metabolism and promotes glucose utilization, protein and lipid biosynthesis by responding to various ligands including growth factors, cytokines and hormones. Activation of this signalling cascade stimulates SREBP transcription factor which regulates fatty acid synthesis and cholesterol-related genes [115,116], and FoxO1, which regulates lipolysis by controlling the expression levels of adipose triglyceride lipase (ATGL) [117]. In vivo evidence indicates that PI3K plays a prominent role in controlling the balance between energy storage and energy consumption. Mice with reduction of PI3K signalling are protected from obesity, hyperglycaemia and liver steatosis. This has been demonstrated in vivo in separate lines of research based on an overall reduction of this signalling cascade or with partial decrease or complete loss of the individual PI3K isoforms [118-123]. Overall, all the evidence published so far, converges to the notion that limiting the activity of the PI3K signalling may be beneficial in cancer treatment but can also safely reduce obesity and improve metabolic syndrome in organisms affected by this condition.

Another important intracellular signalling cascade linking obesity and cancer is the Ras/Raf/MEK/MAPK pathway. This cascade is stimulated by extracellular signals eventually leading to the activation of MAPK [124]. The role of MAPK signalling in obesity-related cancer is partially understood, however, evidence indicates that this signalling cascade can become an important focus of future research on the regulation of metabolism. As mentioned earlier in this review-article, obesity increases the levels of circulating oestrogens, favours hyperinsulinemia and leads to persistent low-grade inflammation. With respect to the cellular mechanisms influenced by these conditions, the mitogenic effects induced by insulin are transmitted through the MAPK/ERK signalling cascade which phosphorylates several transcription factors regulating the expression of genes implicated in the control of the cell cycle and ultimately cell transformation, cancer development and progression [125].

The canonical Wnt signalling cascade by regulating the expression/stability and so the transcriptional activity of the co-factor β-catenin, controls a number of cellular functions (Fig. 1). A plethora of studies have demonstrated the essential role of this signalling pathway in organs development, organism’s homeostasis, cancer and human metabolic disorders including obesity. A comprehensive review on this topic has been published by Chen and Wang [126] where the authors described in great detail i) the distinct roles of this signalling cascade in adipocytes and pre-mature/mature adipocytes, ii) its potential regulation in depot-specific white adipose tissue expansion in response to high fat diet, and iii) its role in the development of depot-specific beige adipocytes. Many questions remain to be answered regarding the precise role of the canonical Wnt signalling in many aspects of the adipose tissue physiology, however, mounting evidence suggests that this signalling cascade plays a crucial role in obesity development and represent a common denominator between deregulated adipose tissue and other chronic diseases in humans including cancer and cardiometabolic disorders [126].

The Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signalling pathway serves as an important downstream mediator of extracellular ligands including cytokines, hormones and growth factors, and transmits a signal to the nucleus where specific transcription factors stimulate gene expression [127]. Animal models and in vitro experiments have been instrumental in identifying the role of the JAK/STAT axis in metabolic organs such as liver, adipose tissue and muscle and the immune system and to show that this cascade regulates glucose tolerance, insulin sensitivity, energy expenditure, adiposity and inflammation [128]. Emerging data have also shown that this pathway is deregulated in cancer and metabolic diseases including obesity and type 2 diabetes [128]. Perhaps, one of the best described molecules linking obesity to cancer via JAK/STAT activation is leptin. Leptin is an adipokine that requires the activation of JAK/STAT for exerting its biological function. By activating this pathway, leptin has been shown to control proliferation, angiogenesis and to promote tumour growth [129]. In addition to adipose tissue, this adipokine as well as its receptor (i.e., OB-R), is overexpressed by breast cancer cells [130] making the leptin/OB-R signalling an attractive target for obesity, cancer prevention and treatment.

Taken all together, there is a growing body of evidence showing that all the aforementioned pathways are implicated in obesity and cancer by regulating cell metabolism, transformation, tumour microenvironment, inflammation and cell immunity and suggesting that implementation of obesity-control could represent an effective strategy to inhibit the growth and metastatic propensity of obesity-related cancers.

Beside the conventional ways to control obesity which include lifestyle changes, surgery and pharmacotherapy, natural bioactive compounds have been increasingly considered to control adipose tissue mass. Not only phytochemicals can control differentiation of pre-adipocytes and adipose tissue growth, they have also been shown to suppress appetite and have anti-inflammatory properties [131] by targeting the pathways related to obesity. A wide range of phytochemicals have been found to reduce obesity and mitigate the effects of obesity related tumours by targeting the aforementioned signalling cascades such as curcumin, resveratrol, tea catechins, and soy phytoestrogens just to name a few [132]. An interesting survey published in 2017 summarized the scientific evidence reported between 2007 and 2017 on the potential use of herbal medicine in the treatment of obesity [133]. The usage of phytochemicals has been proven to be successful in the treatment of many pathological conditions including obesity and obesity-related cancers. However, as mentioned elsewhere in this review-article, there are some limitations linked to their usage such as the possibility of inducing toxicity in liver and kidney, adverse reactions and the often-unknown composition which makes difficult to identify the precise mechanism of action. Nevertheless, the fact that many phytochemicals are known to modulate one or more pathways
associated with cancer development and metabolic diseases like obesity, makes their usage a promising approach for the prevention or control of obesity-related cancers.

7. Limitations of chemo-preventive phytochemicals

Phytochemicals/natural compounds can have positive effects on health. However, there are serious limitations regarding their ability to cure a disease. Multiple risks of harm from herbal ingredients have been documented [134,135]. There is a large scientific literature on traditional herbal medicines, but clinical studies often fail to meet standard methodological criteria, and convincing evidence is often lacking as reported by [136,137]. Most of the natural compound extracts used to treat a disease cannot be assigned to a specific ingredient. As we have seen throughout this review on natural compounds/phytochemicals, these agents show great potential in in vitro tests in cell lines or in animal models using dosages several orders of magnitude higher than the physiological amount obtained from a normal diet. As we have seen in Table 2, the compounds can target distinct signalling pathway(s) such as the PI3K/akt cascade. Yet, all this does not make them applicable for use in patients due to the high concentrations one has to use, if they should show the same beneficial effects seen in vitro. The high dosages are often a result of the low solubility and penetration through the plasma membrane of the cell. High dosages may be toxic in humans and long-term administration of phytochemicals, must be monitored with caution and the risk-benefit weighed before treatment [88]. Flavonoids display anticancer properties. However, they also function as mutagens, pro-oxidants, and inhibitors of drug-metabolizing enzymes [138]. Yet, high consumption of phytochemicals should be considered with caution as mentioned earlier taking into account their dosage, regimes, toxicity, metabolic conversion, transport mechanism, and tissue availability. How can one circumvent the problems associated with high dosage uptake and benefit from the compounds? One approach is to screen libraries to identify natural products against specific targets, e.g., protein kinases.

Beside all the enthusiasm concerning the use of phytochemicals as an improvement for our health there are reports which are warning us. An example is the work by Semba et al., [7] which casts doubts about many reports supporting the idea of resveratrol having a medicinal effect on longevity. The same holds true for curcumin. A plethora of articles has been published on the positive effect of curcumin, a compound isolated from turmeric, yet a scientific prove is still void [139]. The article from Nelson et al., [139] describes curcumin and curcuminoids both as a PAINS (pan-assay interference compounds) and IMPS (invalid metabolic panaceas) compounds. Additionally, many other reports have described the potential dark sides of curcumin questioning its use as an anticancer agent, a therapeutic for Alzheimer’s disease, a treatment for hangover, erectile dysfunction, baldness, hirsutism, a fertility-boosting and contraceptive extract, collectively establishing the properties of a panacea [139].

8. Outlook

(i) Effective compounds should be chosen according to their chemical structure permitting substantial modifications in order to make them soluble and cell-permeable without losing their inhibitory capabilities. (ii) Another option is the targeted delivery of the phytochemicals through new techniques such as multifunctional gold nanoparticles and liposome encapsulation. These methods often increase bioavailability, reduce degradation and enhance therapeutic efficacy [140–147]. (iii) Chemical modification of selected compounds could be made so that their specificity could be down to the nano-molar or even femto-molar range. For these and other reasons, we consider it problematic and wrong that the recently proposed eleventh revision of the International Classification of Diseases (ICD-11) brings some important reforms for popular TCM practices, which lack demonstrable objective and replicable evidence of many chinese herbal medicines. There is a very large scientific literature on TCM herbal medicines, but clinical studies often fail to meet standard methodological criteria, and high-quality evidence is often lacking [136]. Our attempts to focus on the purified well-characterized natural compounds showed that these agents have the potential to interfere with signalling molecules and pathways in vitro. But the challenge for future applications in humans will be to improve their uptake in amounts that can provide a positive consequence for health and minimal side effects.

Author contributions

O.-G.I. conceived the study and wrote a large part of the manuscript. B.G. wrote part of the manuscript, critically revised it and provided financial support. Both authors have read and agreed to the published version of the manuscript.

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Declaration of Competing Interest

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