Pharmacological Effects of Caffeic Acid and Its Derivatives in Cancer: New Targeted Compounds for the Mitochondria

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Abstract

Cancer is a complex pathology of great heterogeneity and difficulty that makes the constant search for new therapies necessary. A major advance on the subject has been made by focusing on the development of new drugs aimed to alter the metabolism of cancer cells, by generating a disruption of mitochondrial function. For this purpose, several new compounds with specific mitochondrial action have been tested, leading successfully to cell death. Recently, attention has centered on a group of natural compounds present in plants named polyphenols, among which is caffeic acid, a polyphenol that has proven to be a powerful antitumoral agent and a prominent compound for studies focused on the development of new therapies against cancer.

In this review, we revised the antitumoral capacity and mechanisms of action of caffeic acid and its derivatives, with special emphasis in a new class of caffeic acid derivatives that target mitochondria by chemical binding to the lipophilic cation triphenylphosphonium.
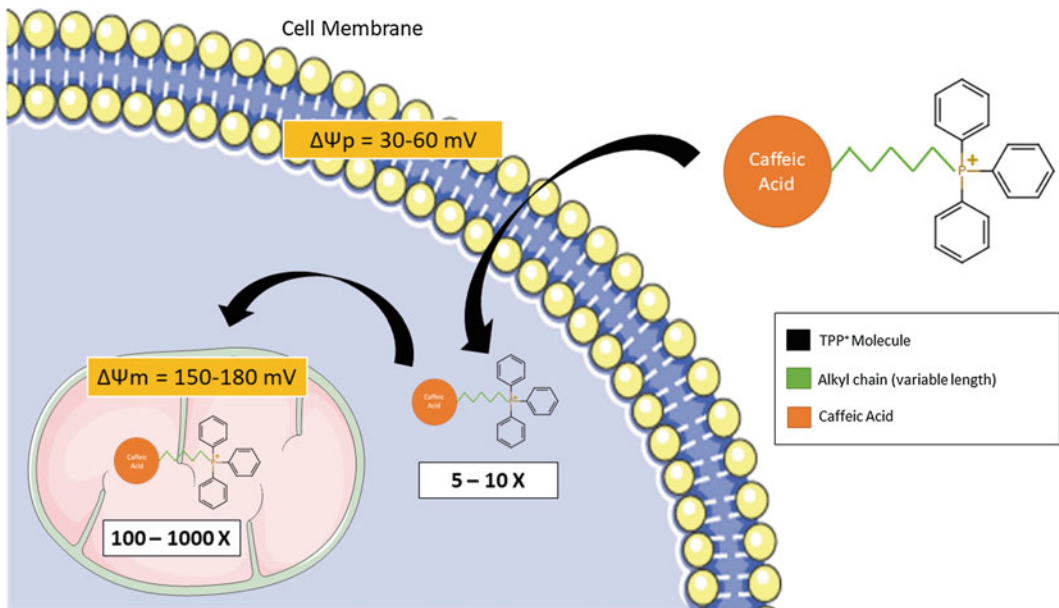
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Graphical Abstract

Abbreviations

α-KG	Alpha-ketoglutarate
ΔΨp	Plasma membrane potential
ΔΨm	Membrane potential of the MII
2-HG	2-Hydroxyglutarate
AMPK	AMP-activated protein kinase
CA	Caffeic acid
CAPE	Caffeic acid phenethyl ester
CAPPE	Caffeic acid phenylpropyl ester
Cyt c	Cytochrome c
DR5	Death receptor 5
DRP-1	Dynamin-related protein 1
ETC	Electron transport chain
FDA	Food and Drug Administration
GSH-Px	Glutathione peroxidase
HIF	Hypoxia-induced factor
IMM	Inner mitochondrial membrane
IC_{50}	Inhibitory concentration 50
MDIVI-1	Mitochondrial division inhibitor 1
MitoCaA	Mitochondriotropic caffeic acid
MitoCA	Mitochondriotropic cinnamic acid
MitoFA	Mitochondriotropic ferulic acid
Mitop-CoA	Mitochondriotropic p-coumaric acid
mIDH	Mutant isocitrate dehydrogenase
MMP	Mitochondrial membrane potential
MMP2	Matrix metalloproteinase 2
MMP9	Matrix metalloproteinase 9
mtDNA	Mitochondrial DNA
mtROS	Mitochondrial ROS
mTOR	Mechanistic target of rapamycin
NFκB	Nuclear factor kappa B
O_2-	Superoxide anion
OH-	Hydroxyl radical
PI3-K/Akt	Phosphoinositide 3-kinase/protein kinase B
p38	Mitogen-activated protein kinase
ROS	Reactive oxygen species
SI	Selectivity indexes
SODs	Superoxide dismutases
TCA	Tricarboxylic acid
TRAIL	Tumor necrosis factor-related apoptosis-inducing ligand
TPP^+	Triphenylphosphonium
VEGF	Vascular endothelial growth factor

1 Background

Noncommunicable diseases represent nowadays the main cause of death in almost every country in the world. Between them, cancer emerges as one of the most significant by reporting an incidence of 24.5 million new cases and 9.6 million deaths in 2017 (Fitzmaurice et al. 2019). In Chile, studies indicate from a total of 106,388 deaths registered in 2017, 27,504 were due to cancer, therefore representing 25.9% of all deaths (Comité Nacional de Estadísticas Vitales 2017) and placing this disease among the pathologies responsible for the largest number of deaths country and worldwide.

Cancer has a great heterogeneity in its origin, course, and response to treatment, which is one of the greatest challenges to achieve, due to different patient response, time-tumor progression, type of tissue, genetic and epigenetic mutations, and metabolic alterations (Prasetyanti and Medema 2017). Due to the high degree of variability, various types of treatments have been developed, chemotherapy being one of the most used of those with clinical efficacy. For instance, main drugs used in chemotherapy are classified according to the type of molecular target and mechanism of action in alkylating agents that cause direct damage to DNA – antimetabolites which correspond to analogs of endogenous molecules that are necessary for DNA and RNA synthesis; mitosis inhibitors which act by altering the formation of the mitotic spindle; and topoisomerase inhibitors that are enzymes that regulate the DNA supercoiling. However, there have been reports of several side effects and drug resistance; therefore, the greatest issue is to improve effectiveness and selectivity against cancerous cells by providing other chemical alternatives. As a result, over the last few years, the search for new pharmacological targets has become relevant in order to develop novel drugs with specific action. Within them, mitochondria have turned out to exhibit special characteristics, allowing the design of selectively targeted drugs (Frattaruolo et al. 2020). As is widely known, this organelle plays a fundamental role in the cell, by participating in vital functions such as ATP production, cycle control, production of metabolic proteins, and cell signaling and death, in addition to being closely related with cellular metabolic stress since it is responsible for reactive oxygen species (ROS) production (Grasso et al. 2020).

2 Mitochondria in Cancer Cells

Cancer cells present various genetic, epigenetic, and metabolic alterations, which lead to the loss of normal cell functions and the acquisition of abnormal survival and proliferation capacities. In this reprogramming phenomenon, the mitochondrion plays a fundamental role by transforming its normal bioenergetic metabolism into an elevated glycolytic metabolism that triggers mitochondrial dysfunction, which is known as the Warburg effect (Grasso et al. 2020; Anderson et al. 2018a).

In cancer, the mitochondria manifest a series of alterations as mutations in mitochondrial DNA (mtDNA), affecting the synthesis of enzymes that participate in the tricarboxylic acid (TCA) cycle and the synthesis of complexes that participates in the electron transport chain (ETC). As a consequence, the production of reductor electron equivalents NADH and FADH2 is altered, resulting in the accumulation of TCA cycle intermediates depending on the affected enzyme. These intermediates can act as oncometabolites, as is the case of fumarate and succinate (Grasso et al. 2020). In addition, tumoral cells present a high rate of ROS production, which is a key feature in carcinogenesis as it correlates with the progression of malignancy, as ROS toxicity may induce the alteration of intracellular pathway signaling (Idelchik et al. 2017). The overproduction of ROS induces the accelerated metabolism and the redox imbalance that cancer cells possess, which prevents the neutralization of ROS. When cells are immersed in hypoxia conditions or a high metabolic rate, electrons can leak from complexes I and III of the ETC and conjugate with O_2 forming a superoxide anion (O_{2-}), which is the main cellular ROS. In this sense, if O_{2-}

cannot be neutralized, it will lead to the oxidation of cellular macromolecules such as DNA, lipids, and proteins (Grasso et al. 2020). As it is well known, ROS neutralization is given by cellular antioxidant mechanisms such as superoxide dismutases (SODs) – which catalyze the reaction of production of H_2O_2 from O_{2-} – and the catalase (CAT) and glutathione peroxidase (GSH-Px) enzymes that catalyze reactions where H_2O is produced from H_2O_2- (Idelchik et al. 2017).

Additionally, mitochondria in tumoral cells are characterized by presenting an abnormal inner mitochondrial membrane (IMM) potential that is higher when compared to normal cells, therefore presenting a more electronegative charge (Kalyanaraman et al. 2018; Modica-Napolitano and Weissig 2015).

3   Antineoplastic Agents Targeting Mitochondrial Function

Given the fact that mitochondria participate in multiple metabolic functions, researchers may employ different strategies to develop more effective drugs, such as targeting the TCA cycle, ETC, mitochondrial biogenesis, or the mitochondrial apoptotic pathway (Frattaruolo et al. 2020; Grasso et al. 2020; Anderson et al. 2018a). Among drugs under study or between those already approved by the US Food and Drug Administration (FDA) are those that operate on the TCA cycle such as CB-839, CPI-613, ivosidenib (AG-120), enasidenib (AG-221), and vorasidenib (AG-881) (Frattaruolo et al. 2020; Grasso et al. 2020; Anderson et al. 2018a, b; Konteatis et al. 2020) (Fig. 1).

In this sense, it has been described that CB-839 is a specific inhibitor of glutaminase. This enzyme converts glutamine into glutamate, in what is supposed to be a physiological process in cells, upregulated in cancer. Excess of glutamate has been associated with an increase in cell proliferation and malignancy (Grasso et al. 2020; Chen and Cui 2015). Additionally, CPI-613 corresponds to a lipoate analog that inhibits the pyruvate dehydrogenase and α-ketoglutarate dehydrogenase enzymes, therefore preventing the incorporation to the TCA cycle of carbons derived from glucose or glutamine (Anderson et al. 2018a; Stuart et al. 2014). Further,
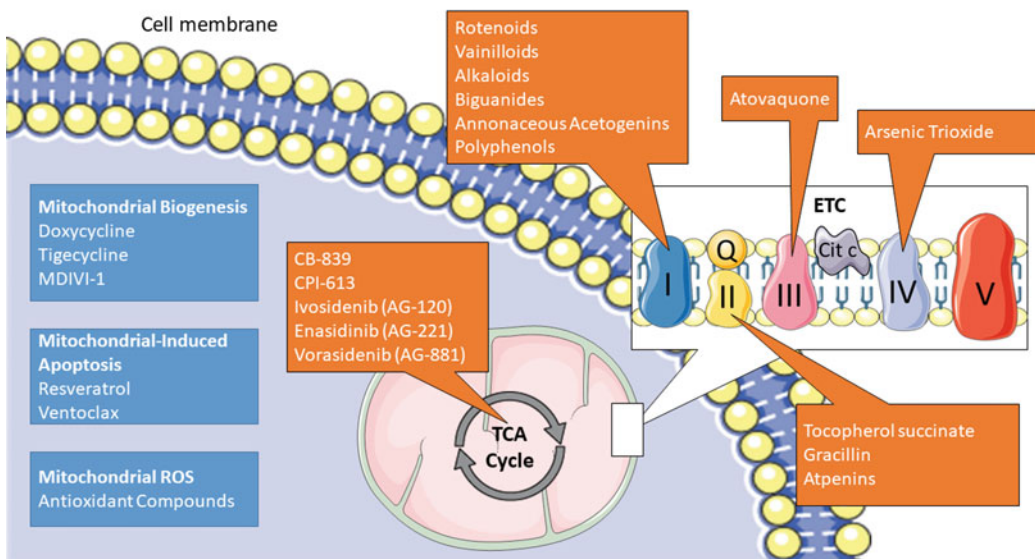

Fig. 1 Agents with antineoplastic effect that act on the mitochondria with action on biogenesis, apoptosis and mitochondrial ROS, TCA cycle, and CTE in their different complexes are described

ivosidenib, enasidenib, and vorasidenib work by inhibiting mutant isocitrate dehydrogenase (mIDH) enzymes. Under normal conditions, isocitrate dehydrogenase should catalyze the conversion of isocitrate to α-ketoglutarate (α-KG); however, when mutated it can catalyze the conversion of α-KG to the oncometabolite 2-hydroxyglutarate (2-HG) (Frattaruolo et al. 2020; Anderson et al. 2018a, b; Abou Dalle and DiNardo 2018). Particularly, ivosidenib inhibits the mutant mIDH1 enzyme, enasidenib inhibits mIDH2, and vorasidenib inhibits both mIDH1 and mIDH2, as described above (Konteatis et al. 2020; Abou Dalle and DiNardo 2018; Popovici-Muller et al. 2018). The mutual mechanism of action consists of blocking the active site that catalyzes the conversion reaction of α-KG to 2-HG (Anderson et al. 2018a, b).

Another strategy that has been developed is to target the different complexes of the ETC (Frattaruolo et al. 2020; Urra et al. 2017; Ashton et al. 2018). As a result, there are several series of drugs described as inhibitors of complex I that can be classified into rotenoids, vanilloids, alkaloids, biguanides, annonaceous acetogenins, and polyphenols (Urra et al. 2017). The latter have been recently attracted attention for their multiple beneficial effects, including antitumoral action (Zhou et al. 2016). Complex I-targeted drugs can inhibit complex I in a competitive or noncompetitive way, being common for competitive compounds to have a hydroquinone/quinone structure, while noncompetitive compounds, such as metformin and other biguanides, can bind non-competitively to different domains of complex I (Frattaruolo et al. 2020). Drugs targeting complex II include α-tocopherol succinate, gracillin, and atpenins. These agents have been described to increase ROS production, leading to apoptosis in cancer cells (Frattaruolo et al. 2020). Among the complex III inhibitor drugs is atovaquone, a ubiquinone analog that can competitively inhibit complex III of ETC as a result of its structural similarity to CoQ10 (Fiorillo et al. 2016). Finally, between drugs whose action is focused on complex IV, the more relevant is arsenic trioxide, a drug used to treat acute promyelocytic leukemia (Ashton et al. 2018).

In addition, researchers have also developed pharmacological compounds interfering with mitochondrial biogenesis, either by preventing the processes of transcription and translation of mtDNA or by altering the fission and fusion dynamics of this organelle (Frattaruolo et al. 2020; Anderson et al. 2018a). Between those that interfere with the DNA-translation process are doxycycline and tigecycline, compounds commonly used as antibiotics that have shown antitumoral effects in many cancer cell lines. As bacteriostatics, they can exert their effect by binding to the 30S ribosomal subunit of bacteria due to the structural similarities between this subunit and mitochondrial 28S ribosomal subunit. Consequently they can block the entry of aminoacyl-tRNA to the A-site of the ribosome, inhibiting the process of elongation and translation of mitochondrial proteins (Dong et al. 2019; Protasoni et al. 2018). Likewise, mitochondrial division inhibitor 1 (MDIVI-1) and indomethacin inhibit mitochondrial fission – an increased process in cancer cells – by blocking dynamin-related protein 1 (DRP-1), which affects mitochondrial dynamics (Frattaruolo et al. 2020; Anderson et al. 2018a; Mazumder et al. 2019).

Another pharmacological strategy is to restore mitochondrial-induced apoptosis, as occurs with resveratrol and venetoclax by inducing mitochondrial release of cytochrome c (Cyt c), therefore initiating the apoptotic cascade by activating the caspase pathway (Frattaruolo et al. 2020; Anderson et al. 2018a).

In recent years, drugs that can inhibit mitochondrial ROS (mtROS) production have been proposed as new strategy, especially those based on antioxidant compounds capable of selectively targeting the mitochondria (Grasso et al. 2020). The accumulation of mtROS produces several alterations in various signaling pathways, activating survival and proliferation factors such as hypoxia-induced factor 1 (HIF-1) and hypoxia-induced factor 2 (HIF-2). As a consequence, it has been described that cells present an elevated angiogenesis and glycolytic enzyme activity that allow the maintenance of ATP production for the tumor cell, despite of increased mtROS (Dickerson et al. 2017). Although excessive

mtROS production is a key process in the development of cancer since it leads to the oxidation of cellular macromolecules (Grasso et al. 2020) and malignant cell transformation, it has been largely observed that very high increases in its production cause the death of cancer cells (Idelchik et al. 2017; Dickerson et al. 2017). However, mechanisms of action of many drugs that causes mtROS accumulation have not yet been thoroughly elucidated.

4   Polyphenols as Antitumoral Agents

Nowadays, the approach of antitumoral therapy focuses on the beneficial effects of polyphenols, a group of compounds that contain two or more phenolic groups. Given their chemical structure, polyphenols have been extensively described as antioxidant, anti-inflammatory, antimicrobial, and antitumoral agents (Zhou et al. 2016). They can be classified into different categories based on their structure and number of phenolic rings, such as stilbenes, lignans, phenolic alcohols, flavonoids, and phenolic acids. This last group can be subdivided into derivatives of hydroxybenzoic acid or hydroxycinnamic acid (Quiñones et al. 2012). Within hydroxycinnamic acid derivatives

is caffeic acid (CA), which exhibits powerful anti-inflammatory, antitumoral, and antioxidant effects, thus controlling oxidative stress by free radicals that constitutes a key process in cancer progression (Caffeic acid 2020). The biological effects exhibited by CA are closely related to its chemical structure. Therefore, its powerful antioxidant effects are associated with the presence of a catechol group in its structure, which is known for its great reducing capacity resulting from two hydroxyl groups (Damasceno et al. 2017).

Antioxidants can be classified as primary or secondary according to their mechanism of action. If they react directly with the radical, they are classified as primary antioxidants, while if they have an indirect effect on radicals, they are classified as secondary antioxidants (Damasceno et al. 2017). CA exhibits both mechanisms. As a primary antioxidant, CA has demonstrated direct neutralization of free radicals by donating protons from its hydroxyl groups to form stable compounds that are not able to produce oxidative damage to cell structures. In the process, CA acquires a semiquinone structure when it has one oxidized group and then an o-quinone structure when both groups are (Damasceno et al. 2017) (Fig. 2). As a secondary antioxidant, CA acts through the chelation of transition metals, which catalyze the decomposition of H_2O_2 in
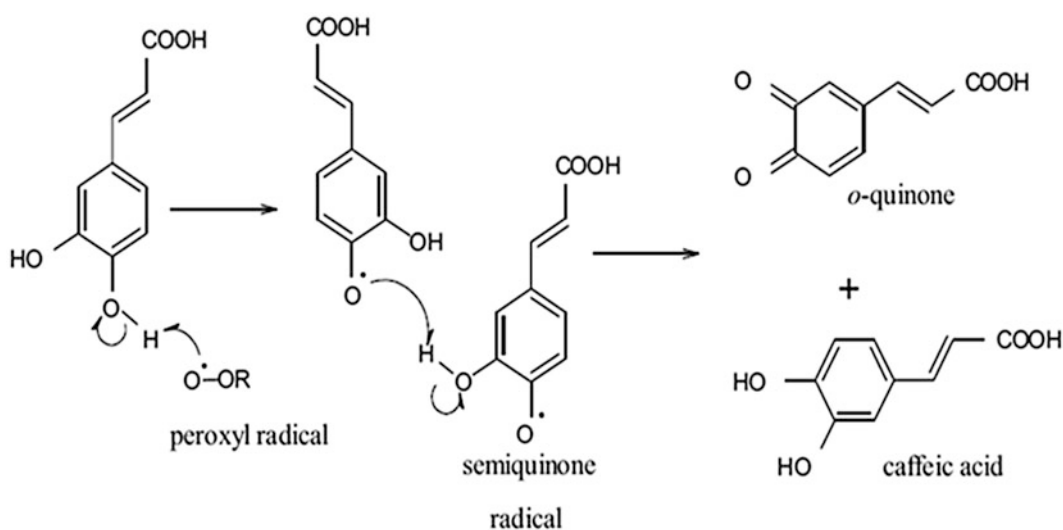

Fig. 2 Possible mechanism involved in the antioxidant activity of caffeic acid. (Damasceno et al. 2017)

hydroxyl radical (OH-). This radical has a great redox potential, which is why it produces oxidative damage in the cell by reacting with lipids, proteins, and nucleic acids (Damasceno et al. 2017). In this process, CA also undergoes structural transformation to semiquinone and o-quinone.

As mentioned above, high concentration CA can behave as prooxidants, and it is mainly to this effect that their effective antitumoral and proapoptotic capacities are associated (Damasceno et al. 2017). Therefore, the administration of high concentrations of CA to cancer cells results in the production of very high quantities of free radicals in presence of O_2 or Cu^{+2}, thus causing extensive oxidative damage and consequently triggering death in tumor cells, without significant side effects on non-cancerous cells, since they have a normal antioxidant balance (Damasceno et al. 2017).

5   Caffeic Acid and Its Derivatives with Antitumoral Action

Numerous studies have shown that CA and its derivatives have proven to be effective on different types of cancer, exhibiting antiproliferative, proapoptotic, antiangiogenic, and antimetastatic effects (Chiang et al. 2014; Monteiro Espíndola et al. 2019; Kabała-Dzik et al. 2018). As in colorectal cancer, CA derivatives such as caffeic acid phenethyl ester (CAPE) and caffeic acid phenylpropyl ester (CAPPE) (Fig. 3) have been described with antiproliferative effects by inducing cell cycle arrest as resulting from the suppression of the mechanistic target of rapamycin (mTOR) and the phosphoinositide 3-kinase/protein kinase B (PI3-K/Akt) signaling pathways (Chiang et al. 2014). Both targets induce cell proliferation and are overexpressed in cancer. The activation of the PI3-K/Akt pathway improves cell proliferation by increasing levels of cyclin D1, which is involved in the progression of the cell cycle from G1 to S phase, hence the antiproliferative effect resulting from the inhibition of this pathway induced by these derivatives.

In addition, CAPE and CAPPE produce an increased activity of the AMP-activated protein kinase (AMPK), which is defined as an energy sensor involved in the maintenance of cellular energy homeostasis. Increased AMPK activity is inversely associated with the risk of cancer by

Fig. 3 Chemical structure of CAPE (**a**) and CAPPE (**b**). (Chiang et al. 2014)
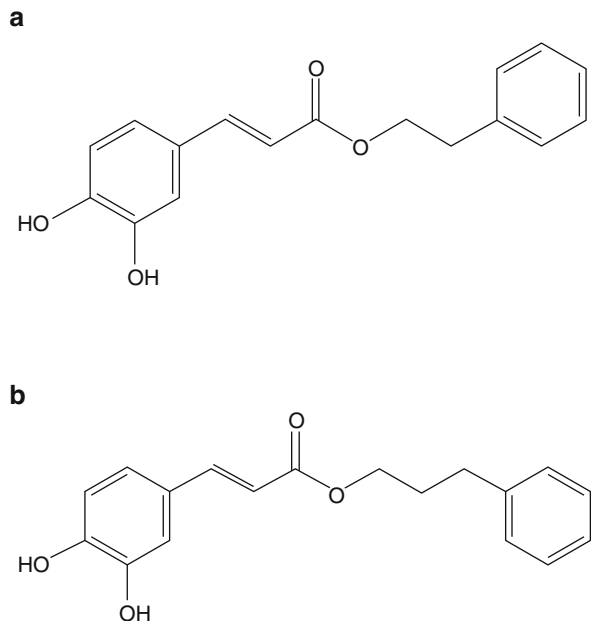
suppressing mTOR activity and increasing apoptosis in cancer cells (Chiang et al. 2014).

As indicated by Monteiro et al., studies in hepatocarcinoma have demonstrated the antiangiogenic and antimetastatic capacity of CA and CAPE. The latter has shown the activation of intrinsic and extrinsic apoptotic pathways (Monteiro Espíndola et al. 2019). In addition, the antiangiogenic capacity of CAPE is given by the inhibition of HIF-1α, which has an increased expression in tumor cells due to the hypoxic environment in which they develop, thus increasing the expression of vascular endothelial growth factor (VEGF) (Monteiro Espíndola et al. 2019). Furthermore, CAPE has an antimetastatic capacity exerted through inhibiting the expression of matrix metalloproteinase 2 (MMP2) and matrix metalloproteinase 9 (MMP9), by suppressing the expression of nuclear factor kappa B (NFκB) (Monteiro Espíndola et al. 2019). These molecules are known for their role in the degradation of the extracellular matrix, which is why they can promote metastasis as a result of their overexpression in cancer.

Additionally, CAPE successfully altered the mitochondrial membrane potential (MMP) in vitro, causing the release of Cyt c from the MMI. This event increases the activation of caspase 9, promoting apoptosis through the intrinsic pathway. Moreover, CAPE also activated the extrinsic pathway of apoptosis that is mediated by the apoptosis-inducing ligand related to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), through upregulation of the death receptor 5 (DR5) resulting from the activation of mitogen-activated protein kinase (p 38) (Monteiro Espíndola et al. 2019).

In studies with breast adenocarcinoma conducted by Kabała-Dzik et al., CA and CAPE have demonstrated a dose- and time-dependent cytotoxic effect, showing greater effects at 48 h rather than 24 h, with a concentration of 100 μM for CA compared to 10 μM for CAPE (Kabała-Dzik et al. 2018). CAPE showed a more powerful cytotoxic capacity than CA, presenting a lower IC$_{50}$ (inhibitory concentration 50) value than the one exhibited by CA (Kabała-Dzik et al. 2018). CA and CAPE also induced the inhibition of

migratory capacity in a dose-dependent manner, showing again that CAPE was more potent than CA (Kabała-Dzik et al. 2018).

6 Mitochondriotropic Derivatives of Caffeic Acid

Given the extensive evidence promoting caffeic acid and its derivatives as powerful antitumoral agents, the idea of creating mitochondria-targeted compounds based on CA emerged to maximize the arrival at their site of action (Teixeira et al. 2018). To achieve this goal, several methods have been developed including those based on compounds linked to triphenylphosphonium (TPP$^+$) (Zielonka et al. 2017).

TPP$^+$ is a lipophilic cation widely used to direct various molecules to the mitochondria since its delocalized positive charge allows selective accumulation within this organelle. This process occurs in two phases. First, the compound enters the cell guided by the electrical attraction generated by a negative plasma membrane potential (ΔΨp) of 30–60 mV. Second, it enters the mitochondria as a result of the even more negative membrane potential of the MMI (ΔΨm), which is between 150 and 180 mV. Therefore, TPP$^+$ acts as a driving force for its accumulation in the mitochondria against the concentration gradient, reaching intracellular concentrations 100–1000 times higher. This feature leads to micromolar range concentrations of these compounds, thus achieving millimolar concentrations within the mitochondria (Zielonka et al. 2017). Furthermore, the incorporation of an alkyl chain serves as a link between TPP$^+$ and the compound of interest, giving different degrees of lipophilicity depending on its length, being more lipophilic with a longer chain length and vice versa (Zielonka et al. 2017) (Fig. 4).

There are only a few studies of CA derivatives linked to a mitochondrial target delivery system such as TPP$^+$. Consequently, Teixeira et al. developed a series of mitochondriotropic antioxidants derived from CA named "AntiOxCINs." These antioxidants are constituted from a primary compound of CA linked to TPP$^+$ which they have
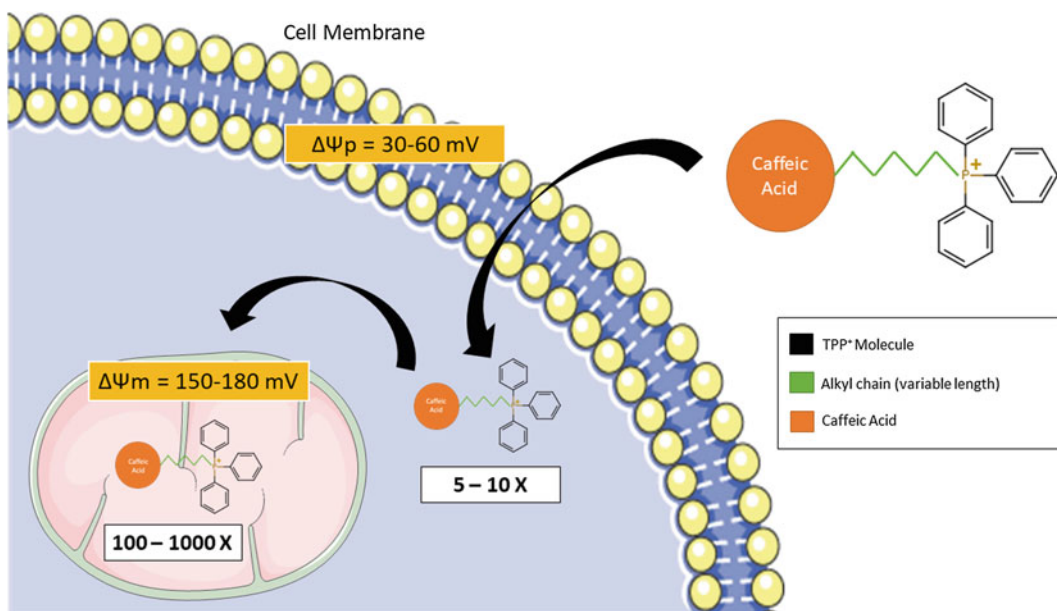
Fig. 4 General structure of TPP$^+$-based mitochondriotropic compounds and their intracellular and intramitochondrial accumulation driven by differences in plasma membrane and inner mitochondrial membrane potential, respectively

called "compound N° 1." Then, they synthetized novel compounds that present the catechol characteristic of CA, named as "compounds N° 22," "N° 23," and "N° 24." In addition, they synthetized another group of compounds derived from pyrogallol group named as "compounds N° 25," "N° 26," and "N° 27." Both groups of new compounds are identical, except for the length of the alkyl chain which can be of six, eight, or ten carbons, as shown in Fig. 5 (Teixeira et al. 2017). The results obtained show that AntiOxCINs present a high chelating capacity on transition metals (even similar to the activity exhibited by EDTA, a known chelating agent), and an increased mitochondrial uptake and ability to prevent mitochondrial membrane lipid peroxidation, compared to compound N° 1. In addition, the compounds were shown to induce the opening of the mitochondrial permeability transition pore (mPTP) (Teixeira et al. 2017), which has a known role in cell death. Its opening causes the mitochondrial release of Cyt c, thus initiating apoptotic signaling pathways (Grasso et al. 2020). Additional tests were performed on compounds N° 24 and N° 25, as they proved to be the most promising

ones. The results illustrated their ability to prevent ROS and Fe^{+2}-related cytotoxicity in HepG2 cells at concentrations of 2.5 μM (compound N° 24) and 100 μM (compound N° 25), within incubation periods of 48 h. In addition, these derivatives did not induce proapoptotic changes related to nuclear morphology or mitochondrial depolarization in normal cells, suggesting their safe use in every type of cells (Teixeira et al. 2017).

Furthermore, in a study developed by Li et al. (2017), mitochondriotropic compounds based on hydroxycinnamic acid derivatives were synthetized, named "MitoHCAs" (Li et al. 2017). The mitochondriotropic character of these compounds was also achieved by binding to TPP$^+$ (Li et al. 2017). In relation to these novel compounds, four new mitochondriotropic compounds based on p-coumaric (Mitop-CoA), caffeic (MitoCaA), ferulic (MitoFA), and cinnamic (MitoCA) acids were synthesized (Fig. 6) and were subjected to several tests evaluating their antioxidant and antiproliferative capacities (Li et al. 2017). The results obtained showed that MitoCaA was the most powerful antioxidant since it inhibits lipid peroxidation
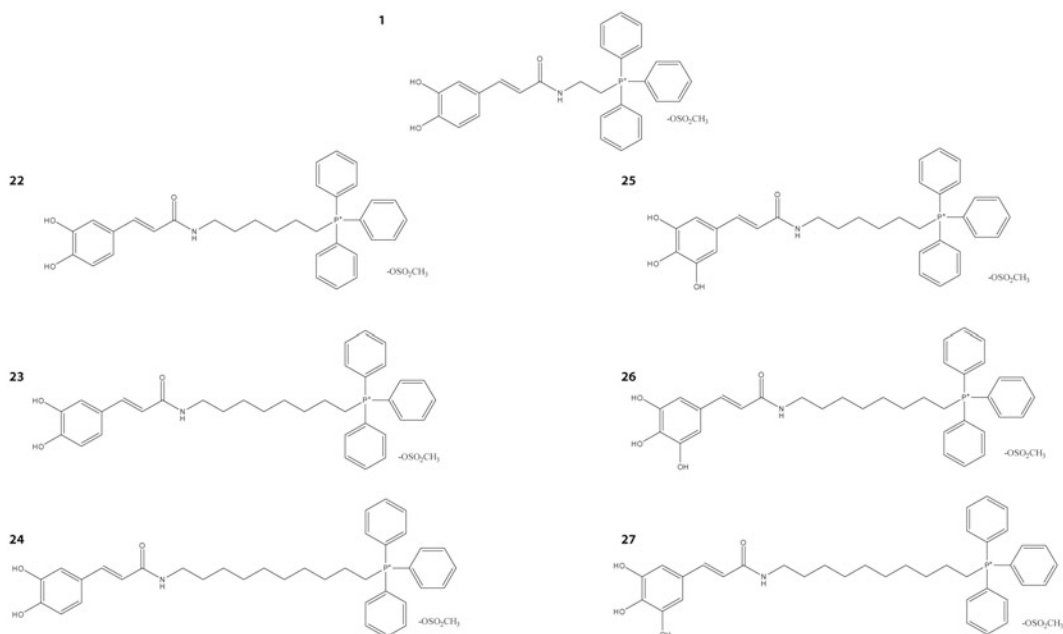
Fig. 5 Chemical structure of the caffeic acid mitochondriotropic derivatives "AntiOxCINs" developed by Teixeira et al. (2017). Compounds Nº 1, 22, 23, 24, 25, 26 and 27
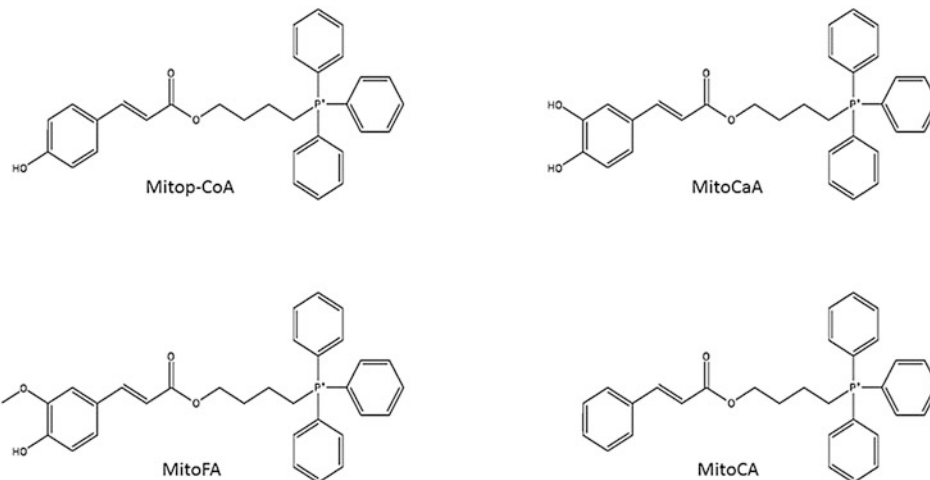

Fig. 6 Chemical structure of the hydroxycinnamic acid derivatives "MitoHCAs" developed by Li et al. (2017)

and exhibits a concentration-dependent behavior (Li et al. 2017). Subsequently, similar results were obtained when comparing the antioxidant capacity of MitoHCAs against endogenous ROS (H_2O_2) (Li et al. 2017). In addition – in order to comprehend the antioxidant mechanism by which MitoHCAs reduce H_2O_2 – results showed that MitoHCAs do not have direct radical elimination capacity and neither exert changes in the production of mitochondrial O_2^-. However, they do

affect the expression and activity of antioxidant enzymes: MitoCaA, MitoFA, and Mitop-CoA increased significantly the activity of the GSH-Px and CAT enzymes, suggesting this is the mechanism by which MitoHCAs decrease H_2O_2 (Li et al. 2017). Furthermore, both MitoCaA and Mitop-CoA were found to have an inhibitory effect on mitochondrial SOD, while MitoFA did not (Li et al. 2017). Additionally, antiproliferative capacity of MitoHCAs against human hepatoma HepG2 cells and normal cell lines (human liver L02 and WI38 diploid human fibroblasts) was also evaluated, resulting in a selective inhibition of cell viability over HepG2 cancer cells compared to normal cells (Li et al. 2017). MitoCA, MitoFA, and MitoCaA compounds demonstrated the most potent antiproliferative capacity against HepG2 cells at 48 h of cell treatment (Li et al. 2017). In addition, selectivity indexes (SI) for MitoHCAs were calculated, which correspond to the quotient between the antiproliferative activity of the compounds in normal cells and the same activity but in HepG2 cancer cells. The compounds with the higher SI were MitoCA, MitoFA, and MitoCaA. The latter was determined as the most selective (Li et al. 2017). Since MitoCaA was the compound with the highest antioxidant and antiproliferative capacity, additional studies were performed. The results showed that MitoCaA caused mitochondrial fragmentation in HepG2 cells in a dose-dependent manner, causing donut-shaped morphology and a discontinuous mitochondrial network at a concentration of 20 μM. Under the same experimental conditions, the compound failed to produce mitochondrial damage in normal L02 cells (Li et al. 2017), although apoptotic assays did demonstrate that MitoCaA possesses a dose-dependent apoptotic effect (Li et al. 2017). MitoCaA was able to induce the apoptosis mechanism through the opening of mPTP and its consequent release of mitochondrial Cyt c (Li et al. 2017). These results differ from the tests performed by Teixeira et al., where it was reported that CA-based mitochondriotropic derivatives would not have an effect on mPTP opening. The difference could be attributed to structural variability in molecular targets and mechanisms of action, despite they originated from the same compound, which requires further studies.

7 Conclusion

Polyphenols and especially CA have been described before as antitumoral, antimicrobial, and antioxidant agents. The novel mitochondriotropic derivatives of caffeic acid have shown to maximize their effects on tumor cells by allowing its selective accumulation within the cancer mitochondria, an organelle on which they exert their effects by altering the redox state and ultimately leading to mitochondrial-mediated apoptosis process. The effects of increased lipophilicity and the mitochondrial targeting product of the link to TPP$^+$ moiety are reflected in more potent compounds than those without TPP$^+$. The selectivity of these compounds for cancer cells is also highlighted, which could be given by the difference in MMP between normal and cancerous cells, the latter exhibiting a higher MMP that leads to the selective accumulation of mitochondrial derivatives. This would indicate a safe use of these compounds, also reducing side effects, a common problem in chemotherapy due to the low selectivity of these drugs. Therefore, in initial studies on tumor cell lines, mitochondriotropic derivatives of CA appear as promising and powerful antitumoral agents for future development of new molecules with targeted approach for cancer therapy, given their increased potency and selectivity.

Supplementary Materials Not applied.

Author Contributions H.B., G.A.V., and M.C. wrote the manuscript. H.B. and G.A.V. did all the figures and table. G.A.V., G.C., J.A.J., and MC edited the manuscript.

Conflicts of Interest The authors declare no conflict of interest.

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