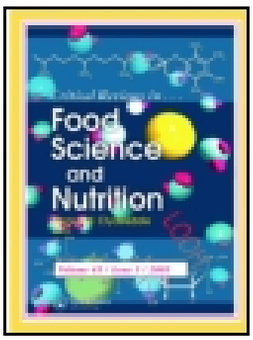

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REVIEW

Natural products targeting into cancer hallmarks: An update on caffeine, theobromine, and (+)-catechin

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ABSTRACT

Natural products have been studied to reveal new therapies against human dysfunctions since they present several medicinal properties. Caffeine, theobromine and (+)-catechin are remarkable natural agents in the class of methylxanthines and flavonoids. These bioactive molecules have several biological activities, for instance, antioxidant, anti-inflammatory, and antitumor capacity. In this sense, studies focusing on these molecules have been performed to discover new treatments against diseases, such as cancer. Cancer is a serious public health problem worldwide responsible for more than 70% of all deaths globally. Industrialized products associated with a sedentary lifestyle and a diet low in antioxidants are related to neoplasms development. Unfortunately, many types of cancers are extremely aggressive and untreatable since, in many cases, they are resistant to chemotherapy. Therefore, revealing new strategies to block cancer growth is one of the biggest challenges to science. In this context, despite the known anticancer actions of caffeine, theobromine and (+)-catechin, it is still essential to elucidate the causal antitumor mechanism of these molecules by analyzing the dysfunctional cancer pathways associated with the hallmarks of cancer. Hence, this review aims to describe the anticancer activity of caffeine, theobromine, and (+)-catechin against the different hallmarks and enabling characteristics of cancer.

KEYWORDS

Cancer; hallmarks; targeted therapy; natural products

Introduction

Noncommunicable diseases (NCDs), i.e. diseases which cannot be transmitted directly from one person to another, are chronic multifactorial disorders responsible for more than 70% of all deaths globally (~ 41 mi per year). Cancer is one of the NCDs and ranks as the second leading cause of death after cardiovascular diseases (WHO 2020a). According to the Global Cancer Observatory (GLOBOCAN) (Globocan, 2020) approximately 19 mi new cases and 10 mi deaths were attributed to this disease worldwide in 2020. The top 5 most frequent cancers (excluding non-melanoma skin cancer) in both sexes were: breast, lung, colorectum, prostate and stomach. Future predictions estimate that by 2040 cancer incidence and mortality in world population will reach 30.2 mi and 16.3 mi cases, respectively (WHO 2020b).

Carcer is a generic term used to describe a large group (> 277) of related diseases which are mainly characterized by an uncontrolled growth of abnormal cells (Hassanpour and Dehghani 2017; WHO 2020a). Virtually any cell in the body can become a cancer cell (carcinogenesis) after suffering a series of successive gene mutations (Hassanpour and Dehghani 2017; NIH 2015). This process occurs in different stages, i.e.: (a) initial stage or initiation, where it has already been identified as genetically altered, (b) promotion stage, where it is considered totally mutated cells, then the malignancy of the cells begins, (c) progression stage, when the tumor is already installed, and as cells start to divide in an accelerated and irreversible way (Siddiqui et al. 2015). Apart from growing locally, cancer cells may also spread to other parts of the body (metastasis), an event considered as a major cause of cancer death (WHO 2020a).

Several genetic and environmental factors are associated to carcinogenesis, including exposure to industrial chemicals, sunlight, air pollution (e.g. particulate matter), some virus infection (e.g. human papillomavirus) and factors related to individual's lifestyle (e.g. tobacco smoking, sedentarism) (Blackadar 2016; Turner et al. 2020). Evidences have been reported that around 90 – 95% of cancers are developed by environmental agents and only 5 – 10% factors are associated to hereditary (Boffetta and Nyberg 2003).

The hallmarks of cancer

The complex nature of neoplastic diseases has always challenged cancer research. Distinct types of cancer can show large differences in terms of genetic alterations, organs affected, impact on systemic physiology, prognostic and therapeutic intervention (Hanahan and Weinberg 2017). In 2000, Hanahan and Weinberg (Hanahan and Weinberg 2000) published a seminal paper which describes some general features encountered in cancers. In this work, they proposed six (later

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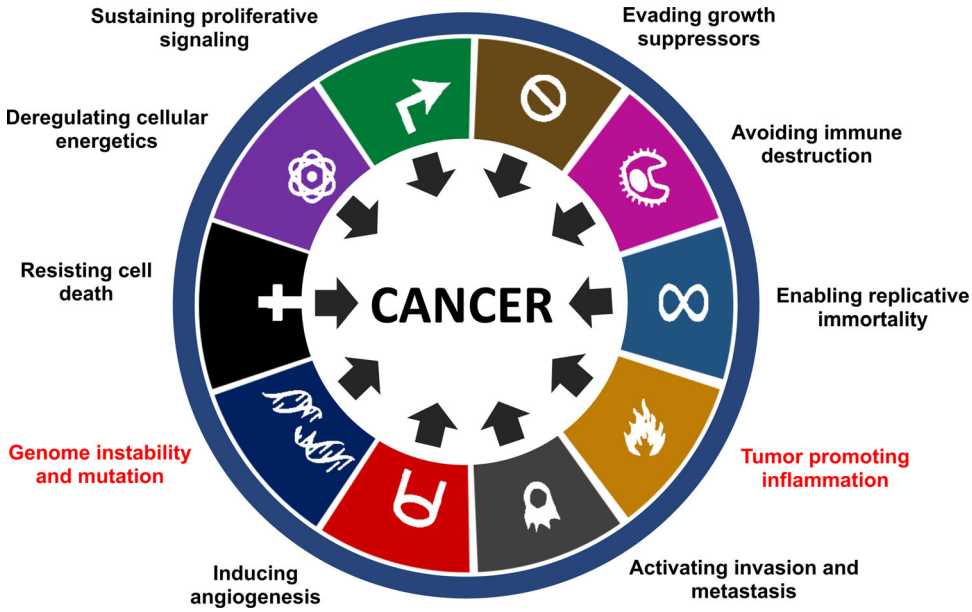


Figure 1. Hallmarks of cancer (in black) and enabling characteristics (in red). Adapted from Hanahan and Weinberg (2011).

extended to eight (Hanahan and Weinberg 2011) capabilities that a normal cell acquires during malignant transformation (a.k.a. the hallmarks of cancer, Figure 1):

Sustaining proliferative signaling – In order to remain in a constant state of proliferation, cancer cells must sustain a proliferative signal. This is mainly achieved by mutations in specific genes (proto-oncogenes) that control proliferative pathways.

Evading growth suppressors – In normal cells, the proliferative signal is counteracted by breaking mechanisms that overrule the initiation of, or block the cell cycle process. These mechanisms are ultimately regulated by tumor suppressor genes (TSGs) which in most cancer cells are dysfunctional due to either genetic or epigenetic alterations.

Resisting cell death – Under normal circumstances aberrant cells undergo programmed cell death (e.g. apoptosis). Cancer cells avoid death using several strategies, such as increasing the expression of antiapoptotic regulators and survival signals and/or downregulating proapoptotic factors.

Enabling replicative immortality – Telomers shorten with each round of cell division. After they reach a certain length, cells are induced to senescence or apoptosis. This mechanism limits the number of times a cell can replicate. Cancer cells circumvent this restriction by avoiding apoptosis or by activating mechanisms that maintain and extend telomeres.

Inducing angiogenesis – Cancer cells demand a continuous supply of oxygen and nutrients as well as metabolic waste removal to keep alive and proliferate. In part, this can be achieved by stimulating the formation of new blood vessels.

Activating invasion and metastasis – High-grade cancer cells tend to become invasive and migratory. They can invade into adjacent tissues and blood/lymphatic vessels reaching other areas in the body where they can start new colonies. The mechanisms underlying invasion and metastasis are complex and may involve both intrinsic and extrinsic (tissue microenvironment) factors.

Deregulating cellular energetics and metabolism – Cell growth and division require a large amount of ATP and molecular building blocks. Cancer cells fulfill their high demands by adapting their energic metabolism using, for instance, aerobic glycolysis and lactate.

Avoiding immune destruction – In some types of cancer, especially those derived from virus infection, cancer cells express antigens for which immune system is not tolerant eliciting an immune response. In order to avoid destruction, cancer cells employ immune-evading mechanisms.

Additionally, they also point out two enabling characteristics that facilitate the acquisition of these hallmarks (Figure 1) (Hanahan and Weinberg 2017):

Genome instability and consequent mutation – The hallmarks capabilities arise in neoplastic cells largely after a series of genomic alterations. Normally, genome integrity is kept by a series of cellular mechanisms that monitor and repair DNA damage as well inactivate/intercept mutagenic molecules. If the damage is irreparable, cells are eliminated via apoptosis. Neoplastic cells may show defects in different components of these mechanisms and as they proliferate more mutations may occur. In most cases, this results in cell death, but some mutant phenotypes can be advantageous allowing them to undergo clonal expansion.

Tumor-promoting inflammation – Virtually all neoplastic lesions are infiltrated by different types of immune cells, collectively known as infiltrating immune cells (IIC). These cells may induce an inflammatory response which paradoxically contributes to the arising of several cancer hallmarks in incipient neoplastic cells. In part, this occurs due to the release of proliferative, pro-survival and proangiogenic factors by IIC.

Current therapies for cancer treatment

Today there are several therapeutic strategies used in cancer treatment. The most common include surgery, chemotherapy, radiotherapy, targeted therapy, immunotherapy, stem cell or bone marrow transplant and hormone therapy (American Cancer Society (ACS)), 2020). Some of them act locally in the

body (e.g. surgery and radiotherapy) whereas others are systemic (e.g. chemotherapy and targeted therapy). They can be administrated solely or in combination with other therapies (e.g. radiotherapy plus chemotherapy) and vary according to the type of cancer and its stage (NIH 2020).

Chemotherapy and small molecule targeted therapy represent two therapeutic approaches which tackle cancer using chemical compounds. Essentially, chemotherapy drugs are cytotoxic agents that interfere in different phases of cell cycle. Their application is justified by the idea that cancer cells generally have a higher division rate than normal ones and, thus they tend to be more chemosensitive. These drugs can be divided into five main biochemical classes: alkylating agents (e.g. cisplatin), anti-metabolites (e.g. 5-fluoroacil), anti-tumor antibiotics (e.g. doxorubicin), topoisomerase inhibitors (e.g. topotecan) and tubulin-binding drugs (e.g. paclitaxel) (Dickens and Ahmed 2018; American Cancer Society (ACS)), 2019). Despite their efficacy, chemotherapy drugs can also affect normal cells causing significant adverse effects, such as nausea and vomiting (Rapoport 2017), alopecia (Rossi et al. 2017), mucositis (Cinausero et al. 2017), myelosuppression (Epstein et al. 2020) and peripheral neuropathy (Kerckhove et al. 2017). Moreover, they are also associated to multidrug resistance (MDR), an undesirable phenomenon responsible for over 90% of deaths in cancer patients under chemotherapy (Bukowski, Kciuk, and Kontek 2020).

In contrast to chemotherapy, small molecule targeted therapy (SMTT) relies on chemical compounds that modulate specific molecular targets in cancer cells. These targets are genetically modified in cancer and are essential to tumor development and survival. In most cases they are involved in signaling pathways which are dysregulated during carcinogenesis (Ke and Shen 2017; Lee, Tan, and Oon 2018). Examples of target-oriented compounds include some drugs administered in clinics, such as inhibitors of tyrosine kinases (e.g. imatinib), proteasome (e.g. carfilzomib), cyclin-dependent kinase (e.g. ribociclib) and poly ADP-ribose polymerase (e.g. niraparib) (Lee, Tan, and Oon 2018). Due to their specificity, SMTT drugs are expected to be less toxic to healthy cells, though side effects have been reported (e.g. rash, diarrhea, hypertension) (Bashraheel, Domling, and Goda 2020; Lee, Tan, and Oon 2018; S. Liu and Kurzrock 2014). Additionally, they also may stimulate mechanisms that lead to drug resistance (Boumahdi and de Sauvage 2020). The limitations of the current SMTT drugs, as well as chemotherapy drugs, reflect an urgent need for new anticancer compounds that better balance efficacy/toxicity and avoid drug resistance.

Natural products as anticancer agents

Natural products (e.g. plants, minerals and animal derived) have been used in the treatment of human diseases since the ancient times (Calixto 2019; Dutta et al. 2019). Even today, they are still potential sources for drug development, as demonstrated in a recent review (Newman and Cragg 2016): from all 1211 new worldwide small-molecule approved drugs in 1981-2014 period, 51% were composed of natural-product based compounds. They are prescribed to treat several diseases, mainly cancer, bacterial infections and hypertension. In the same paper, a more extensive research (1940s–2014) revealed that natural-based products represented 68% of all 136 small-molecule anticancer drugs available in this period.

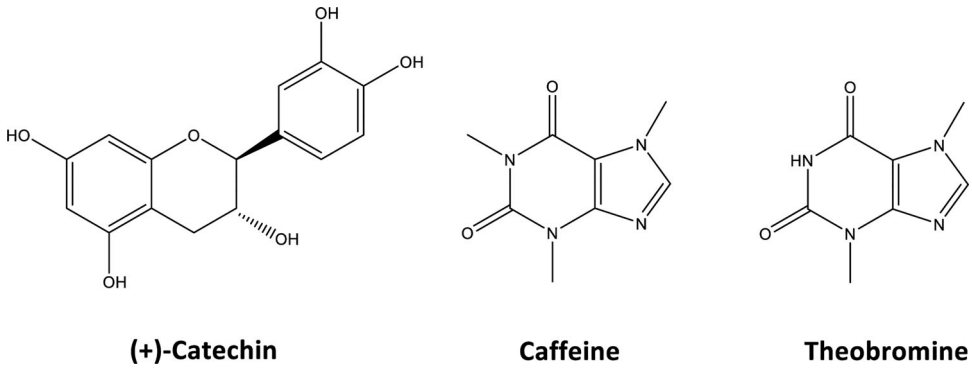
Plants are one of the main natural sources of compounds with biological activity (Majolo et al. 2019). Today there are around 350,000 registered species of vascular plants in the world and every year new ones are considered (Royal Botanic Gardens 2020). This represents a vast and yet underexplored area for drug discovery. From a therapeutic perspective, plants can be used in many different forms such as teas, extracts and dyes. Moreover, their active compounds can be isolated and used as medication or serve as precursors for synthetic/semi-synthetic drugs (Majolo et al. 2019). Indeed, the list of phytochemicals (i.e. chemical compounds produced by plants) with therapeutic activity is extensive and contains a variety of chemical classes including alkaloids, terpenes, flavonoids, essential oils, gums and several primary and secondary metabolites (Priya and Satheeshkumar 2020).

The anticancer properties of phytochemicals have been widely studied aiming their application in some modalities of cancer treatment, such as chemotherapy and targeted therapy (Majolo et al. 2019; Efferth et al. 2017; Barabadi et al. 2017; Efferth and Oesch 2021). Phytochemical cytotoxic activities have been demonstrated in different types of cancer and in in vitro/in vivo cancer models (e.g. cultured cells and xenografts). They are proposed to act through several mechanisms, including: induction of cell cycle arrest and apoptotic/non-apoptotic cell death, inhibition of tumor angiogenesis, signal transduction, invasion and metastasis, as well preventing carcinogenesis (Majolo et al. 2019; Efferth and Oesch 2021). Apart from standalone treatment, phytochemicals can also participate as adjuvants in cancer chemotherapy through three possible ways: enhancing drugs efficacy (e.g. chemosensitization), reducing chemoresistance (e.g. inhibiting MDR) and relieving adverse effects (e.g. reactive oxygen species (ROS) scavenging) (Lin et al. 2020).

Some investigations (Cadoná et al. 2017; Martin, Goya, and Ramos 2013) reported that extracts that present a chemical matrix rich in bioactive molecules, such as guaraná (*Paullinia cupana*) and cocoa (*Theobroma cacao*), have anticancer effects. A possible justification is the presence of bioactive compounds, such as flavonoids and methylxanthines (Cadoná et al. 2017; Martin, Goya, and Ramos 2013). These compound classes are best known for their antioxidant and central nervous system (CNS) stimulating properties, but there are plenty of reports on their anticancer activity in cellular or animal models. Accordingly, in the first part of this review, we will discuss the nature and biological activities of some phytochemicals from flavonoid ((+)-catechin) and methylxanthine (caffeine and theobromine) chemical classes (Figure 2) focusing on their anticancer properties.

The biological effects of caffeine, theobromine, and (+)-catechin

Regular consumption of natural products, such as teas, fruits, vegetables and whole grains, has been associated with a lower risk of chronic diseases, for example, heart disease, cancer, stroke, diabetes, Alzheimer's disease, cataracts, and functional decline related to age (Liu 2013; Kozłowska and



(+)-Catechin **Caffeine** **Theobromine**

Figure 2. Chemical structures of (+)-catechin, caffeine and theobromine.

Szostak-Wegierek 2014). An estimative of 30-35% of death causes related to cancer are associated with poor diet in the US (Daniele et al. 2017). Studies suggest that increasing the intake of natural products is a good strategy to prevent chronic diseases. This can be explained by the significant presence of phytochemicals in these functional foods (Liu 2013; Kozłowska and Szostak-Wegierek 2014).

The Dietary Guidelines for Americans (USDA 2015) recommend a healthy eating pattern, which includes consumption of a good source of vegetables, for example, dark green, red, and orange, vegetables (beans and peas), starch; fruits, mainly whole fruits; grains, at least half of which are whole grains; fat-free or low-fat dairy products; a variety of protein foods and oil. Thus, modification of diet and lifestyle with the inclusion of plant foods plays an important role in controlling the development of chronic diseases (Liu 2013; Daniele et al. 2017). Among the phytochemicals that are present in natural products and are able to promote health benefits, caffeine, theobromine, and (+)-catechin are highlighted in this area, since they have been negatively correlated with the risk of developing dysfunctions (Isemura 2019; Monteiro et al. 2019).

(+)-Catechin is a representant of flavonoids. Flavonoids (from the Latin word *flavus* which means yellow) represent one of the most important and diverse groups among products of natural origin. This group is composed of low-molecular-weight polyphenolic substances which are synthetized in plants as secondary metabolites (Panche, Diwan, and Chandra 2016; Sandu, Bîrsă, and Bahrin 2017). Flavonoids are often responsible for the pigmentation of flower petals, but they also contribute to plants flavor, growth, reproduction and defense (Kopustinskiene et al. 2020; Sandu, Bîrsă, and Bahrin 2017).

All flavonoids have in their structure a basic flavan skeleton, a 15-carbon phenylpropanoid chain (C6-C3-C6 system), which forms two aromatic rings (A and B) linked by a heterocyclic pyran ring (C). They are gathered into six different major groups according to their chemical structure, degree of oxidation and linking chain unsaturation: isoflavonoids (e.g. genistein), flavanones (e.g. hesperetin), flavanols (e.g. (+)-catechin), flavonols (e.g. quercetin), flavones (e.g. apigenin) and anthocyanidins (e.g. cyanidin) (Kopustinskiene et al. 2020) (Figure 3).

These natural compounds are largely distributed in the human diet. Anthocyanins may be found in fruits and flowers, whereas flavanols in fruits, teas, hops, nuts, and coconut water. Flavones and flavonols are basically found in citrus fruits, leaves, and isoflavonoids in vegetables, especially soy. Besides, flavanones are frequently found in branches, stem, leaves, roots, flowers, fruits. They are considered extremely important as natural protectors of the organism against several dysfunctions, such as coronary heart disease, myocardial infarction, cancer, neurodegenerative psychic diseases, and other chronic diseases (Guven, Arici, and Simsek 2019; Procházková, Boušová, and Wilhelmová 2011).

Flavonoids perform several known biological and therapeutic activities (Horáková 2011; Ayaz et al. 2019). However, the ones that stand out the most are the antioxidant properties. Flavonoids antioxidant activities can occur through a direct or indirect way and depend on their number of hydroxyls. This activity allows flavonoids to inhibit or delay the oxidation processes generated by ROS (Jucá et al. 2020).

Taking this into account, (+)-catechin presents these biological properties described above. A review conducted by Isemura (2019) emphasized the beneficial effects of (+)-catechin in human health. This study reported that this bioactive molecule has a remarkable antioxidant activity that can act against several chronic-degenerative dysfunctions, such as cancer, cardiovascular and neurodegenerative diseases.

Moreover, caffeine and theobromine are considered important methylxanthines. There are historic and anthropologic evidence that methyxanthines have been include in human diet since a long time ago. Currently, the regular intake of these substances is frequently present in human population (Monteiro et al. 2019).

Methylxanthines also present important biological activities, which have several benefits for human health, acting against respiratory and cardiovascular diseases, cancer, obesity and diabetes, human infertility, neurological and neurodegenerative diseases (Monteiro et al. 2019). Methylxanthines are natural products produced by plants and many of them are used to prepare drinks for human consumption, the most popular are coffee, tea and cocoa (Franco, Oñatibia-Astibia, and Martínez-Pinilla 2013).

Methylxanthines (or methylated xanthines) are heterocyclic organic compounds derived from xanthine, a purine base that plays a very important role in the catabolism of nucleotides and a precursor of uric acid (Monteiro et al. 2019; Singh et al. 2018). Their chemical structures contain two fused rings (pyrimidinedione and imidazole) which are

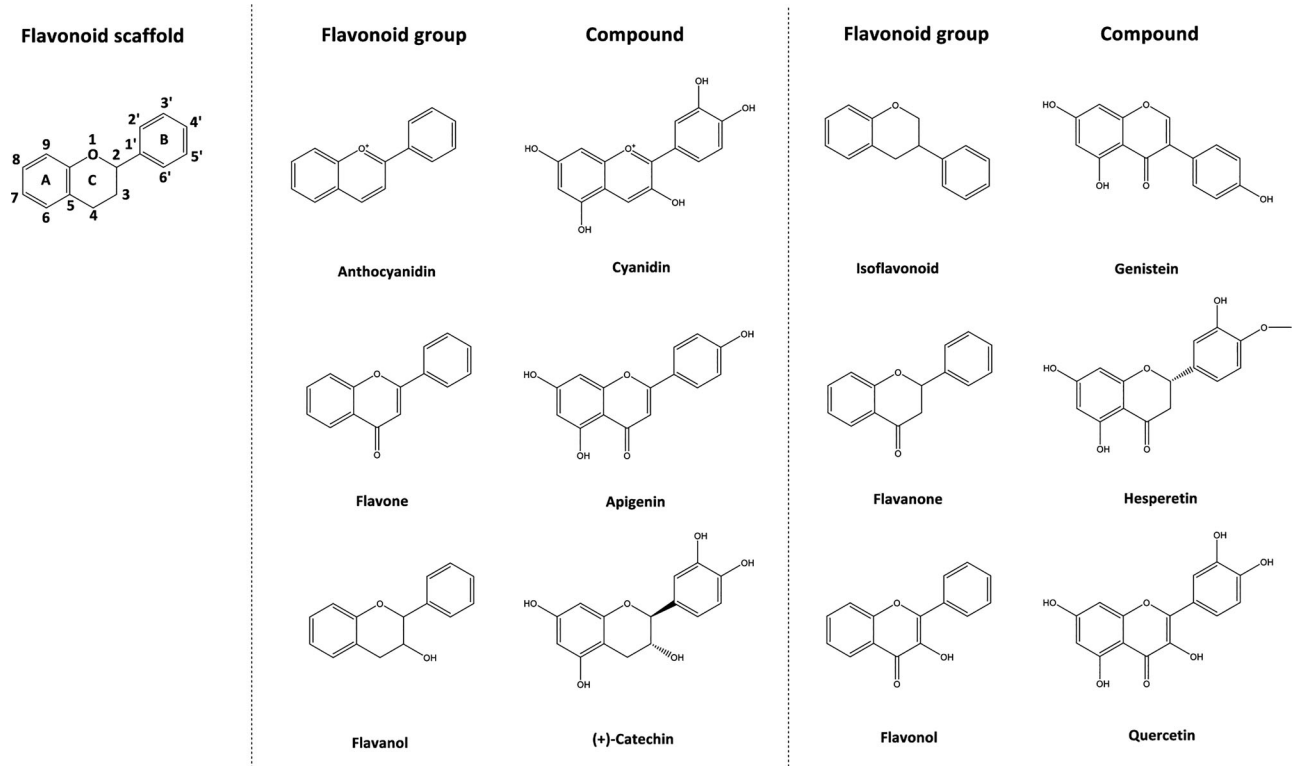


Figure 3. Chemical structures of six major groups of flavonoids.

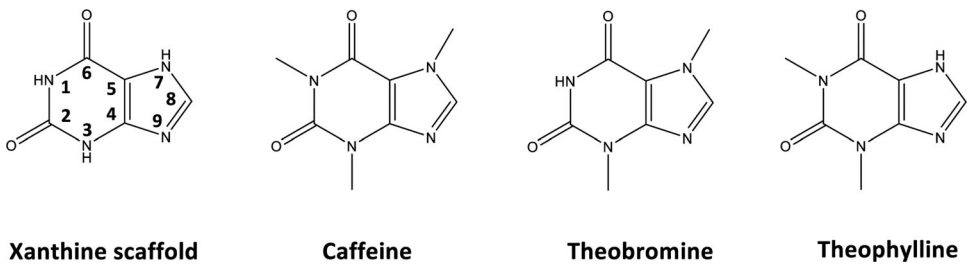


Figure 4. Chemical structures of main methylxanthines.

substituted with methyl groups (Monteiro et al. 2019; Singh et al. 2018) (Figure 4). In plants, these molecules have a protective role against pathogens and insects (Monteiro et al. 2016; Schuster and Mitchell 2019). The most abundant natural methylxanthines include caffeine (1,3,7-trimethylxanthine), theobromine (3,7-dimethylxanthine) and theophylline (1,3-dimethylxanthine), which are commonly consumed by humans in the form of teas (*Camellia sinensis* var. *siensis* and *Camellia sinensis* var. *assamica*), coffee (*Coffea* sp.), guaraná (*Paullinia cupana* sp.), mate (*Ilex paraguariensis*) and cacao (*Theobroma cacao*) (Monteiro et al. 2019; Monteiro et al. 2016). The molecular mechanisms of methylxanthines include inhibition of cyclic nucleotide phosphodiesterase, mobilization of intracellular calcium and also antagonist of adenosine receptors (Franco and Ware 2019).

The biological effects exerted by flavonoids and methylxanthines have been extensively investigated (Monteiro et al. 2019; Panche, Diwan, and Chandra 2016). Some of them have potential therapeutic application as antiviral (e.g. anti influenza), anti-inflammatory, antioxidant, anti-diabetes, cardioprotective and anticancer agents (Jucá et al. 2020). Among the most important bioactive molecules, caffeine and theobromine from methylxanthines class and (+)-catechin, a flavonoid, can be highlighted by their remarkable medicinal properties, emphasizing a potential anticancer activity (Xu et al. 2020; Sugimoto et al. 2014; Guruvayoorappan and Kuttan 2008). Some examples of their anticancer properties are shown in Table 1.

Taking this into consideration, health benefits of caffeine and theobromine are well established in the literature. A review carried out by Monteiro et al. (2019) reported that these molecules can act as Central Nervous System stimulants, bronchodilators, coronary dilators, diuretics, and anticancer adjuvant treatments. Also, they present positive effects against neurodegenerative diseases, cardiovascular problems, diabetes, and fertility.

Bioavailability of caffeine, theobromine, and (+)-catechin

The biological benefits of caffeine, theobromine, and (+)-catechin depend on its bioavailability in the organism as

Table 1. Anticancer activities of caffeine, theobromine and (+)-catechin.

Bioactive molecule	Reference	Target Cancer	Model	Effective doses/concentrations
Caffeine	Chen, Chou, et al. 2014	Brain	RT-2 malignant glioma cells	0.5 mM
	Okano et al. 2008	Liver	Human liver cancer cells (HCC)	10 mM
	Rosendahl et al. 2015	Breast	MCF-7 and MDA-MB-231 cell lines	5 mM
	Cadoná et al. 2016	Colorrectal	HT-29 cell line	1.22 μg/mL
	Eini et al. 2015	Melanoma	Rats	Drinking ad-libitum water containing caffeine (0.1% wt/vol)
	Ku et al. 2011	Brain	U87MG cell line	20 μM
	Wang et al. 2019	Hepatocellular	Hep3B cells / Subcutaneous xenograft tumor model was established by transplanting SMMC-7721 cells into nude mice.	0.5 and 1 mM / 20 mg/kg/day, every other day for 2 weeks
	Maugeri et al. 2018	Glioblastoma multiforme	Glioma cells	2.5; 5; 10 mM
	Saiki et al. 2011	Cervical	HeLa cell line	10 and 25 mM
	Tsabar et al. 2015	Cervical	HeLa cell line	50 mM
	Cadoná et al. 2017	Breast	MCF-7 cell line	0.0012; 0.012; 0.12; and 1.2 mg/mL
	Miwa et al. 2011	OsteosarcomaFibrosarcoma	MG63 cell lineHT1080 cell line	0.25, 0.5, 1, 2.5; 5 mM
	Liu, Zhou, and Tang 2017	Gastric	MGC-803 and SGC-7901 cell lines	0.5, 1, 2, 4 and 8 mM
	Miwa et al. 2012	Osteosarcoma	HOS cell line	0.5; 5; 1 mM
	Tej et al. (2019a)	Fibrosarcoma	Carcinogen-induced tumor model using mice	Drinking water containing 0.02%, 0.04%, or 0.08% w/v caffeine
	Tej, Neogi, and Nayak (2019b)	Melanome	B16F-10 melanoma injected in C57BL/6 mice	Drinking water (0.08% w/v, daily)
	Liu and Chang (2010)	Leukemia	U937 cells	10; 50 and 100 μg/mL
	Chen, Chou, et al. (2014b)	Brain	Rat and human glioma cells	0.5 mM
	Merighi et al. (2007)	Colorectal	HT-29 cell line	1 and 10 μM
	Sarkaria et al. (1999)	Lung	A549 cell line	0.01; 0.1; 1 and 10 mM
	Lin et al. 2014	Cervical	HeLa cell line	1; 2.5; 5; 10 and 15 mM
Theobromine	Cadoná et al. 2016	Colorectal	HT-29 cell line	0.67 μg/mL
	Sugimoto et al., 2014	Glioblastoma multiforme	U87MG cell line	3 and 10 mM
	Barcz et al. 1998	Ovarian	Ovarian cancerous cells	20 μg/mL
	Gil et al. 1993	Lung	E14/W lung carcinoma cells injected in BALB/c mice.	1-125 mg/kg body weight
(+)-Catechin	Cadoná et al. 2016	Colorrectal	HT-29 cell line	0.43 μg/mL
	Guruvayoorappan and Kuttan 2008	Melanome	B16F-10 melanoma injected in C57BL/6 mice	5; 25 μg/mL
	Sun et al. 2020	Lung	A549 cells	200; 400 and 600 μmol/L
	Manikandan et al. 2012	Colon and liver	HCT15, HCT116, and HepG-2 cell lines	50 μM
	Park 1999	Human myeloid leukemia	U937 cell line	8–50 μM

is the absorption, distribution, and elimination. Moreover, indirect effects of diet on intestinal physiology (pH, intestinal fermentation, bile excretion, time of intestinal transit, microbiota) are also considerable factors in the absorption of these bioactive substances. Besides, interactions between these bioactive molecules and other food components, such as proteins and polysaccharides found in the food matrix, can interfere with their absorption (Kamiloglu et al. forthcoming; Arnaud 2011).

Kamiloglu et al. (forthcoming) accomplished a review about the effect of co-ingestion of flavonoids, such as (+)-catechin, with other macro- (carbohydrates, lipids, and proteins) and micro-constituents (vitamins, minerals, and other micronutrients) in foods. This study reported that proteins, dietary fiber, and minerals are associated with an unfavorable effect on the bioavailability of flavonoids. However, lipids, digestible carbohydrates, vitamins, alkaloids, carotenoids, and other flavonoids can increase flavonoid bioavailability.

In addition, a review carried out by Arnaud (2011), indicated that methylxanthines, such as caffeine and theobromine, are able to achieve all body fluids and can cross all biological membranes. These bioactive molecules are metabolized by the liver and do not deposit in organs or tissues. Dietary components, for example, broccoli, herbal tea, and alcohol can interact with caffeine and alter its plasma pharmacokinetics.

Moreover, Sorrenti et al. 2020 reported that cocoa bioactive molecules, such as theobromine and (+)-catechin, can present bioavailability modulation depend on the individual microbiota. When these bioactive molecules are available in the intestine, they interact with gut microbiota to become better absorbed in the intestine, since only its secondary bioactive metabolic can reach the systemic circulation and achieve organs to produce their biological activities. Therefore, gut microbiota alterations, as known as dysbiosis, can directly affect the bioavailability of these substances."

Anticancer activity of caffeine, theobromine and (+)-catechin

Caffeine is a methylxanthine that is highly present in drinks, such as coffee, tea, cocoa and guaraná. Its chemical resemblance with adenosine allows caffeine to modulate the responses mediated by adenosine receptors in different

organs (van Dam, Hu, and Willett 2020). Well-known effects include enhancement of mood and alertness and improvement of cognitive functions (e.g. awareness and reaction time) and physical performance (Souza et al. 2017; Tej and Nayak 2018; van Dam, Hu, and Willett 2020). Other important biological functions include antioxidant and anti-tumor activities. The chemopreventive effects of coffee, that is rich in caffeine, are being widely explored, among these are inhibition of oxidation, anti-inflammatory effects and induction of cellular apoptosis of cancer cells (Xu et al. 2020).

Carcinogenesis is an evolutionary process trigging DNA mutation, directly related to high levels of ROS, which can induce the oxidation of lipids and proteins, favoring DNA mutation. Previous studies reported that caffeine acts inhibiting DNA repair in cancer cells, promoting cell death, and also in modulating important events involved in cancer development, such as angiogenesis and metastasis (Tej and Nayak 2018; Xu et al. 2020).

Theobromine is another bioactive methylxanthine found in large amounts in cocoa beans and which may be responsible for several beneficial effects associated with chocolate intake (Jang et al. 2018). It has been described to act as a respiratory stimulant agent, smooth muscle relaxant, vasodilator, myocardial stimulant and diuretic. The main mechanisms of action of theobromine include phosphodiesterases inhibition, blockage of adenosine receptors and stimulation of the CNS (Cova et al. 2019; Debnath et al. 2018; Smit, 2011).

Theobromine plays a role similar to caffeine in the face of cancer pathology, mainly in the interference of tumor angiogenesis (Song et al. 2009). This methylxanthine has been studied for some years as a possible drug in chemotherapy treatments. In the study by Skopinska-Rozewska et al. (Skopinska-Rózewska et al. 1998), they aimed to deepen an investigation evaluating the effect of theobromine on mouse cutaneous angiogenesis. Their results indicated a reduction in the formation of new blood vessels, which may prevent the risk of metastasis.

The preventive activity of (+)-catechin, an important flavonoid, against cancer is being extensively investigated (Cadoná et al. 2016; Guruvayoorappan and Kuttan 2008; Manikandan et al. 2012; Sun et al. 2020). This compound is found in large quantities in different types of fruits, red wine, green tea and chocolate. Its polyphenolic structure promotes electron delocalization, neutralizing ROS molecules and promoting a remarkable antioxidant action (Yang and Wang 2016).

Methylxanthines and flavonoids can modulate processes and biochemical pathways involved in carcinogenesis, as well as act as modifiers of biological responses, supporting and maintaining the immune system and protecting cells from damage generated by ROS (Niedzwiecki et al. 2016). In a nutshell, these molecules can interfere with all cancer hallmarks and their enabling characteristics. In the second part of this review, we will describe in more details the molecular mechanisms that underlie these effects.

Caffeine, theobromine and (+)-catechin targeting into cancer hallmarks

Hallmark 1: Sustaining proliferative signaling

Normal cells can control cellular growth by external stimuli that modulate proliferative pathways. Cancer cells develop some strategies to dysregulate these pathways and sustain proliferative signaling. Among several pathways that are modified in cancer cells, the mitogen-activated protein kinases (MAPK)/extracellular signal- regulated kinases (ERK) pathway represents an important role in this cancer hallmark (S. Lee, Rauch, and Kolch 2020).

MAPK pathway allows cells to interpret a wide variety of external signals, triggering or affecting different biological events, such as gene expression, mitosis, metabolism, survival, apoptosis and differentiation. It is suggested that the MAPK signaling pathway is involved in anti-apoptotic processes and that its activation guarantees cancer cells greater survival advantage, such as apoptosis evasion (Cargnello and Roux 2011).

Members of the MAPK family include different molecules, such as ERKs 1/2, c-Jun N-terminal kinase (JNK) 1, 2, 3 and p38 protein (p38α, p38β, p38γ, p38δ). These molecules can be activated by ROS production (Finkel 2011). ERKs signaling is involved in several mechanisms, for example, in cell proliferation modulation, which is stimulated by activating factors of mitosis, promoting the advance of the G1 and S phases of the cell cycle. In this sense, ERK plays a crucial role in carcinogenesis, since it can promote survival, migration, angiogenesis, cell cycle, cell development and differentiation (Krishna and Narang 2008). In addition, studies have reported that many ERK route inhibitors are deregulated in cancer, promoting a continuous expression of proteins in this pathway, contributing to increase of proliferation and survival of tumor cells (Dhillon et al. 2007). Moreover, p38 protein is essential to promote cell proliferation and ensure cell survival. This molecule is frequently deregulated in tumor processes since its activation promotes accelerated growth and cell survival (Zheng et al. 2015).

MAPKs are responsible to control expression of cyclins, which are molecules essential to sustaining the cell cycle. Studies have shown that inhibitors of MAPKs blocked cyclins activity, and consequently, promoted cell death (Terada et al. 1999). Therefore, cyclins are molecules that also are dysregulated in cancer cells. For instance, D cyclins (D1, D2 and D3), that coordinately function as allosteric regulators of cyclin dependent kinase 4 and 6 (CDK4/CDK6), present a crucial role to regulate cell cycle transition from G1 to S phase. Overexpression of cyclin D1 promotes a deregulation in CDK activity, resulting in rapid cell growth under conditions of limited mitogenic signaling, without cellular checkpoints, stimulating neoplastic proliferation. Besides, upregulation of cyclin D1 sequestrates the proapoptotic protein BAX (BCL2-associated X protein) in the cytoplasm, in this way increasing Bcl-2 (B-cell lymphoma 2) antiapoptotic action (Beltran et al. 2011). In this sense, investigations have been performed to reveal new anti-cancer agents targeting into cyclins blocking.

Therefore, inhibitors of this signaling route may be used in anti-cancer therapies. In this context, studies have shown important actions of caffeine by inhibiting cancer growth via blockage of sustaining proliferative signaling and, consequently, increasing cancer cell death. Okano et al. (Okano et al. 2008) suggested that caffeine has an antiproliferative action on human hepatocellular carcinoma cells (HCC). They reported that caffeine blocked the HCC growth by cell cycle arrest at the G0/G1 phase, however, this was not associated to apoptosis. Moreover, this study revealed that caffeine promoted the activation of MEK/ERK pathway. This mechanism generated downstream up-regulation of epidermal growth factor receptor (EGFR), a transmembrane receptor tyrosine kinase associated to signal transduction pathways that modulate cell growth and apoptosis, via MEK/ERK/EGFR signaling.

One of us (Cadoná et al. 2017) has reported that caffeine produced cytotoxicity against breast cancer cells line (MCF-7) by inhibiting MAPKs pathway. Caffeine was able to inhibit MAPKs pathway in MCF-7 cells via reduction of p-p38 and its substrate expression, p-HSP27. Moreover, Maugeri et al. (2018) reported that caffeine presented anti-cancer action in glioblastoma multiforme (GBM) cells line. Caffeine was able to decrease cellular proliferation by inhibition of MAPK/ERK signaling pathway.

Chen et al. (Chen, Chou, et al. 2014) also report that caffeine presented cancer antiproliferation capacity against malignant glioma cells line (RT-2). This study suggested that caffeine modulated cell cycle and increased RT-2 malignant glioma cells death by caspase-dependent and caspase-independent apoptosis pathways. Besides, this investigation indicated that caffeine promoted the induction of glioma cell death by increasing eukaryotic initiation factor 2 (eIF2) phosphorylation, which results in repression of protein translation, decreasing cyclin D1 expression, inducing cell cycle arrest at G1 and G0/G phases.

Further, Rosendahl et al. (Rosendahl et al. 2015) analyzed the cytotoxicity of caffeine in positive estrogen receptor (ER+) (MCF-7) and negative estrogen receptor (ER-) (MDAMB-231) breast cancer cells. Caffeine decreased the growth of ER + and ER- cells by reducing ER and cyclin D1 abundance in ER + cells. These effects promoted impaired cell cycle progression and cell death activation.

Sun et al. (Sun et al. 2020) showed that (+)-catechin presented anti-cancer activity against non-small cell lung cancer A549 cells. The findings suggested that (+)-catechin presented cytotoxicity in A549 lung carcinoma cells by induction of cyclin kinase inhibitor p21 and suppression of cyclin E1 in a dose-dependent manner, resulting in cell cycle arrest. Also, theobromine presented anti-cancer activity in malignant glioma cell line (U87-MG) (Sugimoto et al. 2014). The results showed that theobromine increased intracellular cAMP levels and stimulated the activity of p38 mitogen-activated protein kinase and c-Jun N-terminal kinase. This causal mechanism plays an important antiproliferative role against U87-MG cells.

A summary of the action of these bioactive molecules targeting into proliferative signaling is shown in Figure 5.

Hallmark 2: Evading growth suppressors

Among different genes that are dysregulated in cancer cells to evade growth suppressors, *p53* is perhaps the most prominent. Some situations of cellular stress, such as exposure of cells to radiation or chemotherapeutic agents, can induce apoptosis through DNA damage, involving the tumor suppressor *p53* gene. When genotoxic stress is generated, *p53* is activated promoting cell cycle arrest in G1 phase, to repair genomic injury. If the DNA is not restored, *p53* triggers cell death by activating apoptosis. Therefore, this mechanism ensures that cells with mutated DNA do not remain in the organism, preventing cancer development (Jeggo, Pearl, and Carr 2016). However, *p53* is frequently mutated or absent in most cancer cells. In this sense, *p53* is not able to play its correct role and with that, genetically altered cells proliferate, favoring the appearance of cancer (Vijayakumaran et al. 2015).

A previous study reported that caffeine and (+)-catechin were able to up-regulate *p53* in colorectal cancer cells (HT-29), promoting cell cycle arrest and decreased cell proliferation levels (Cadoná et al. 2016). Moreover, Liu et al. (Liu, Zhou, and Tang 2017) reported that caffeine presented antiproliferative effect on gastric cancer cells (MGC- 803 and SGC-7901) in vitro. These findings were associated with caffeine ability to upregulate *p53* in both cell lines, promoting apoptosis. Also, Miwa et al. (Miwa et al. 2012) suggested that caffeine upregulated *p53* in human osteosarcoma cell line (HOS), activating apoptosis.

Hallmark 3: Avoiding immune destruction

The immune system presents a crucial role to avoid cancer development by several mechanisms. However, sometimes mutated cells can escape from this system and promote cancer development (Mittal et al. 2014). In this sense, several studies have been conducted to develop immunotherapy against cancer. These immunotherapies are based on checkpoint blockade. There are antibodies that block inhibitory receptors, for example CTLA-4 and PD-1, that reduce immunologic responses to avoid excessive tissue damage in the setting of immune response, and thus promote antigen-specific immune responses to eliminate cancer cells. Likewise, adenosine signaling decreases inflammatory response to protect the tissues against intense immunologic response that could cause tissue injury. Adenosine in the immune microenvironment unleashes the activation of the A2a receptor, which is considered one such negative feedback loop, stimulating the production of anti-inflammatory cytokines, such interleukin-10 (IL-10), and reduces secretion of pro-inflammatory cytokines, such as tumor necrosis factor- α (TNF- α), IL-12, interferon gamma (IFN-γ) and IL-2, and thus suppressing immunologic cells activity. Since adenosine is present in high concentrations in the tumor microenvironment, blocking A2a receptor activation plays a crucial role to enhance anti-tumor immunity (Leone, Lo, and Powell 2015).

Since the activation of the immune system is a field intensely investigated to promote cancer destruction, it is

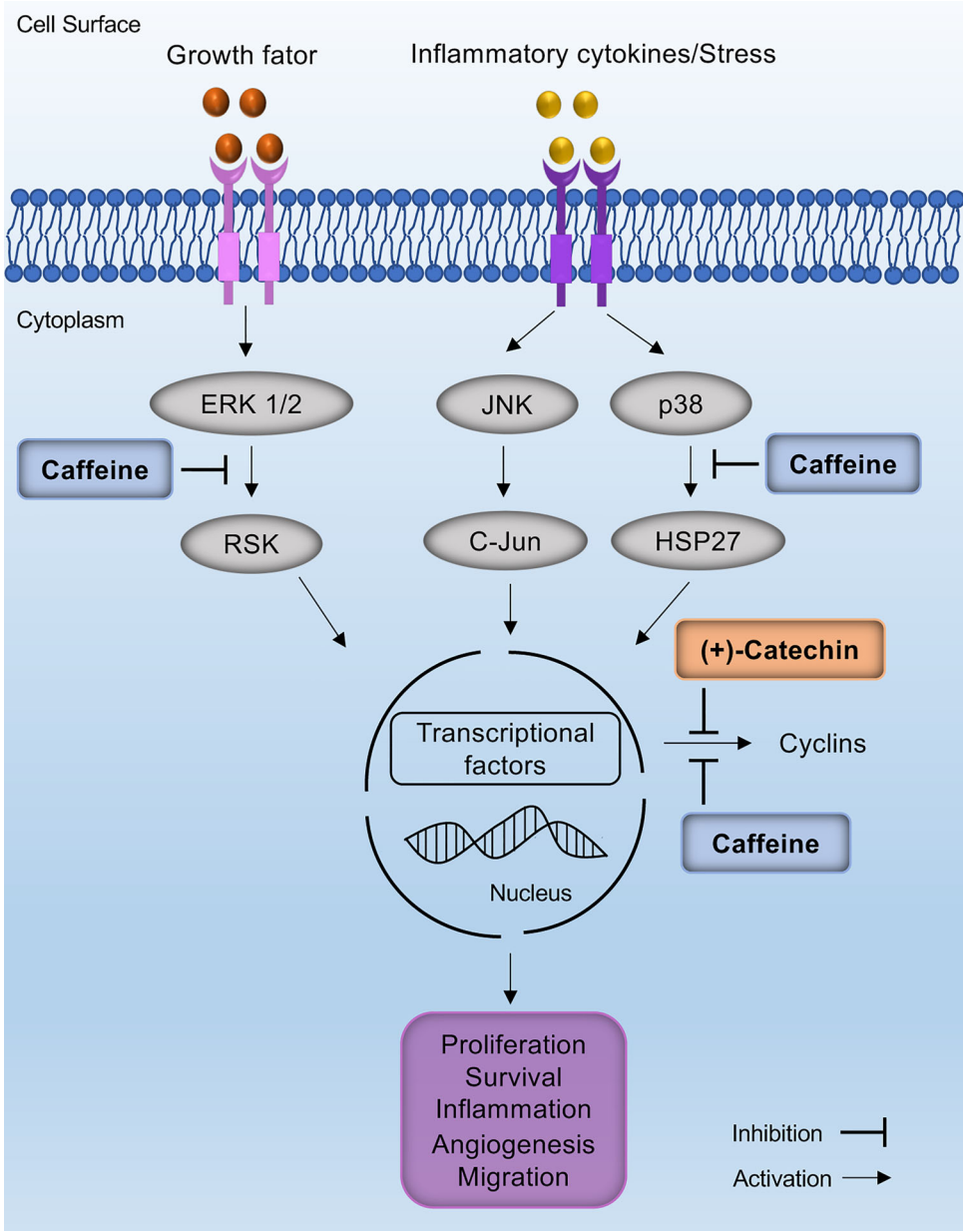


Figure 5. Molecular targets of caffeine and (+)-catechin in MAPKs pathway. Arrows indicate increased expression or activation. T bars indicate inhibition or reduced activity.

very important to reveal new therapies to target this point. In this sense, Eini et al. (Eini et al. 2015) investigated the role of caffeine as antagonist of A2aR by activating antitumor immunity. The action of caffeine was analyzed in wild-type and A2aR knockout (A2AR-/-) mice. In particular, it was investigated the antitumor effect of caffeine in carcinogen 3-methylcholanthrene (3-MCA)-induced transformed cells or B16 melanoma cells inoculated into animal footpads. The results indicated that caffeine-consuming mice presented tumors developing at a lower rate compared to water-consuming mice. Moreover, the findings showed that caffeine promoted the activation of antitumor immunity by increasing leukocyte infiltrates, composed primarily of lymphocytes, which were more abundant in samples of caffeine-treated mice than those found in water-consuming mice.

In a similar way, A2aR-/- mice presented reduced rates of 3-MCA-induced tumors. Caffeine treatment inhibited tumor growth and increased proinflammatory cytokine release when compared to water-consuming mice. The addition of the adenosine receptor agonist, NECA, decreased IFN-γ levels whereas the exposition to ZM241385, a selective A2aR antagonist, increased IFN-γ levels. Therefore, immune response modulation through either caffeine or genetic deletion of A2aR leads to a Th1 immune profile and inhibition of carcinogen-induced tumorigenesis.

A previous investigation also reported that theobromine was able to block A2aR receptor using intradermal inoculation of E14/W lung carcinoma cells in BALB/c mice. The results suggested that theobromine reduces neovascularization, tumor progression and metastasis, via A2aR inhibition (Gil et al. 1993).

Moreover, another study showed that (+)-catechin was able to increase IL-2 in B16F-10 melanoma injected in C57BL/6 mice, reducing tumor growth. Since IL-2 presents

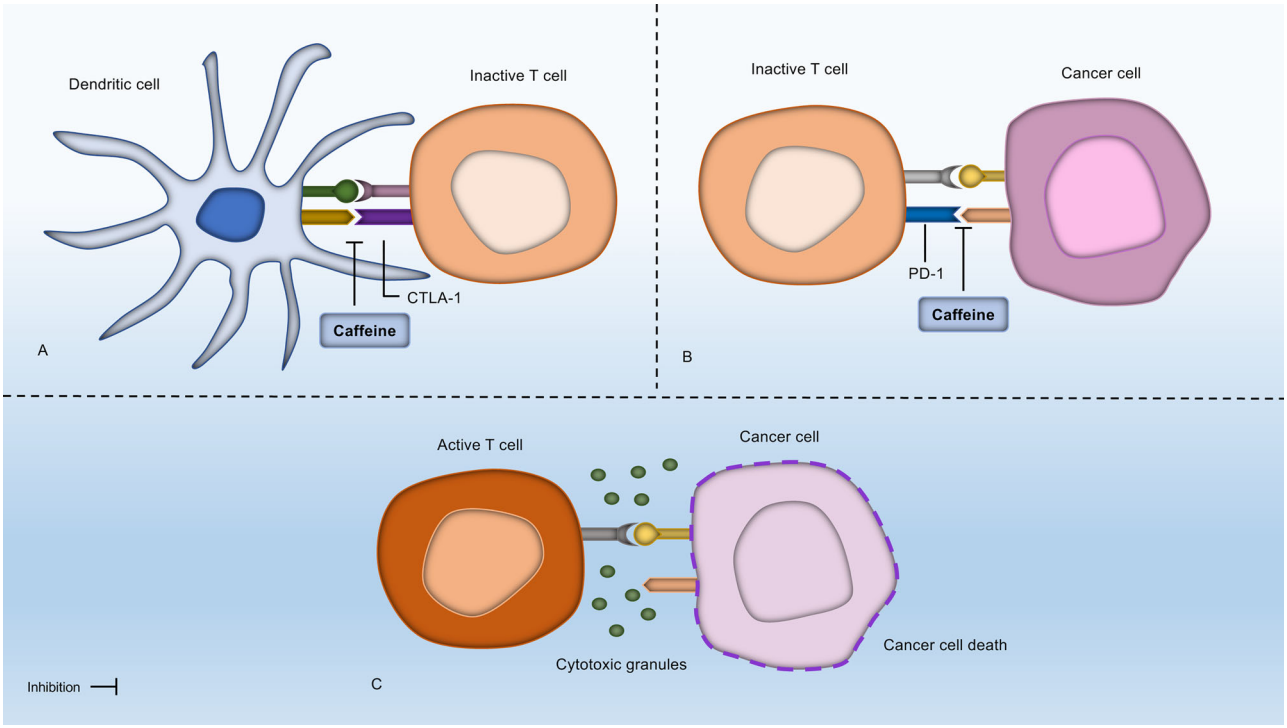


Figure 6. Caffeine effects on immune system. A) Caffeine can block the immune system checkpoint, CTLA-1, that prevents dendritic cells from priming T cells to identify cancer cells. B) Caffeine can inhibit the immune system checkpoint, PD-1, that prevents T cells to destroy cancer cells. C) Active T cells, after CTLA-1 and PD-1 inhibition by caffeine, attacking cancer cells. T bars indicate inhibition or reduced activity.

a crucial role to the activity of the natural immune system, the activation of this molecule can stimulate natural killer cell and cytotoxic T-lymphocyte production. In this sense, (+)-catechin can induce the immune system against tumor development (Guruvayoorappan and Kuttan 2008).

Other studies showed some evidence that caffeine was able to inhibit PD-1 receptor, increasing immunity response and TNF-α and IFN-γ levels, thus promoting cancer cells death. In this context, Tej et al. (Tej et al. 2019a) analyzed whether caffeine could affect T cell infiltration into the tumor and expression of PD-1 receptor on T lymphocytes during tumor development in a carcinogen-induced tumor model. The findings indicated that caffeine decreased tumor incidence as well as tumor growth levels. However, caffeine-treated groups decreased the infiltration of CD4 + CD25+ regulatory T lymphocyte. Moreover, caffeine reduced the PD-1 expression on CD8+ T lymphocytes and CD4 + CD25+ regulatory T lymphocytes. Also, caffeine modulated cytokines production by increasing TNF-α and IFN-γ levels.

Since anti-PD1 monotherapy presents low complete response levels, showing reduced efficacy, studies have been performed to reveal new immunotherapeutic combinations that can improve anti-tumor immunity. In this context, Tej et al. (Tej, Neogi, and Nayak 2019b) reported that caffeine enhanced anti-tumor effect of anti-PD1 monoclonal antibody. The results indicated that synergism of caffeine and anti-PD1 mAb improved the antitumor action against B16F10 melanoma tumors. Also, this combination therapy indicated additive increase in infiltration of CD4+ and CD8+ T lymphocytes into the B16F10 melanoma tumors. On the other hand, combined therapy indicated a reduction in infiltration of CD4 + CD25 + T regulatory cells. In addition, caffeine and anti-PD1 mAb synergism presented higher intra-tumoral TNF-α and IFN-γ levels.

Figures 6 and 7 summarize the findings described for avoiding immune destruction (hallmark 3).

Hallmark 4: Enabling replicative immortality

Telomeres are repeated sequences of non-coding DNA (TTAGGG), which provide stability to chromosomes. Most cells divide about 50 times then become senescent and activate apoptosis to be eliminated. This cellular aging process is called the Hayflick limit. With each cell division, telomeres are shortened, thus telomeres can indicate when cells are already senescent and need to stop dividing and activate the apoptosis (Gaascht, Dicato, and Diederich 2015).

Telomerase is an enzyme that plays an important role to avoid telomeres loss and thus senescence process. This enzyme is active only in stem cells. However, mutated cells frequently present this molecule activated to evade apoptosis and become immortal, which is a significant hallmark in cancer cells. This alteration can unleash other pathway dysfunctions that can enable replicative immortality, such as tumor colony formation, cell cycle acceleration and apoptosis evasion (Gaascht, Dicato, and Diederich 2015).

Since alteration in telomerase promotes tumor colony formation, a previous study investigated the action of caffeine to inhibit this process. In this context, Ku et al. (Ku et al. 2011) investigated the anti-cancer action of caffeine in gliomas in vitro, using U87MG cell line, as well as in vivo, using athymic mice that received U87-MG cells injected subcutaneously into their right flank. This study focused on

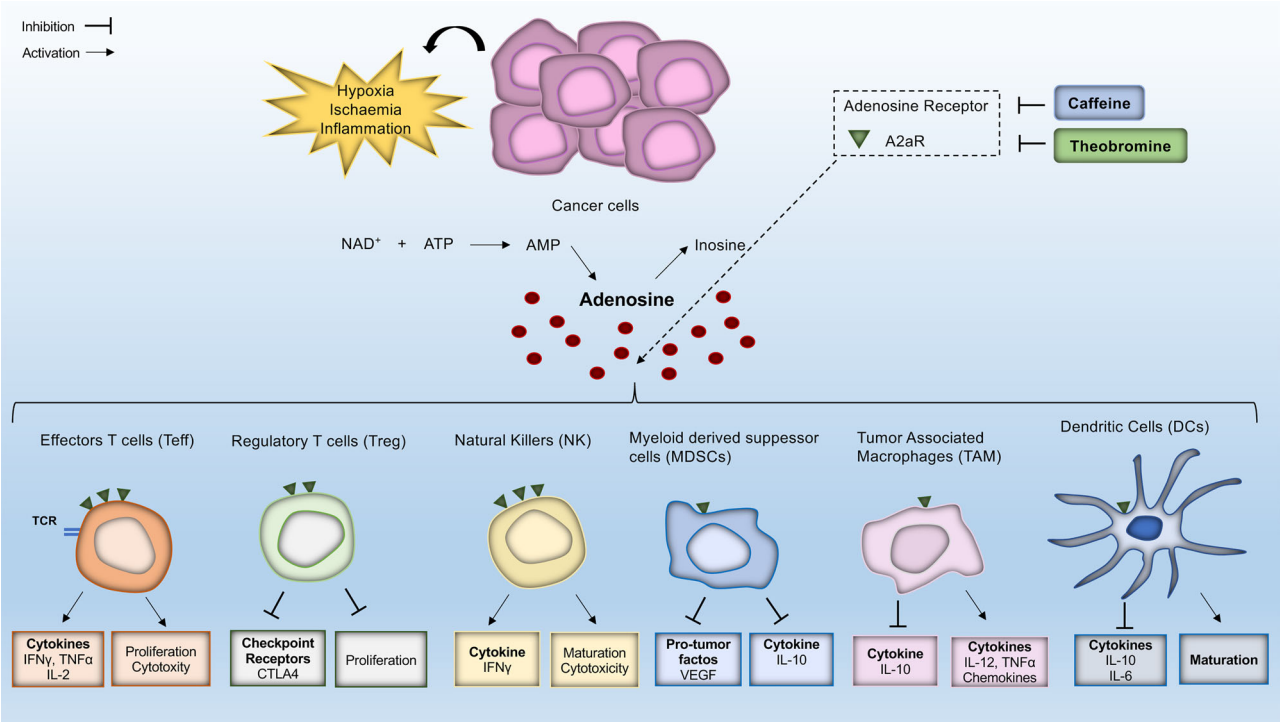


Figure 7. Production of adenosine in tumor microenvironment promotes immunosuppression and tumor development. Caffeine and theobromine can modulate immune pathways by inhibiting adenosine receptors and thus, activate immune cells to recognize and destruct cancer cells. Arrows indicate increased expression or activation. T bars indicate inhibition or reduced activity.

the causal mechanism by analyzing replicative immortality inhibition. The results found in vitro suggested that caffeine blocked cellular proliferation and reduced colony formation. Moreover, caffeine treatment promoted G0/G1-phase cell cycle arrest by suppressing Rb retinoblastoma protein (Rb) phosphorylation. Moreover, caffeine activated apoptosis through caspase-3 activation and poly (ADP-ribose) polymerase (PARP) cleavage. Besides, caffeine phosphorylated serine 9 of glycogen synthase kinase 3 beta (GSK3β). Cells were pretreated with a pharmacological inhibitor of protein kinase A (PKA), H89, that was capable to antagonize caffeine induced GSK3βser9 phosphorylation. This result indicated that the mechanism should be associated to cAMP-dependent PKA-dependent pathway. The results found in the in vivo experiment suggested that caffeine-treated tumors showed decreased proliferation and stimulated apoptosis when compared with vehicle-treated tumors.

Hallmark 5: Activating invasion and metastasis

Cancer presents a natural pattern to spread to the organism and to colonize other tissues, an important cancer hallmark based on activating invasion and metastasis. These tumor features are associated with many molecules and pathways that allow cancer cells to move via blood and lymphatic vessels to healthy tissues, such as epithelial – mesenchymal transition (EMT) and matrix metalloproteinases (MMPs). MAPKs pathway is responsible for activating AP-1 transcription factor that regulate MMP-2 and MMP-9 expression in cancer cells (Liu and Chang 2010).

Also, hypoxia presents a considerable impact on solid tumors, since this event contributes to the proliferation and metastasis of cancer cells. Hypoxia- induced invasive and migratory capacity promotes the upregulation of MMP- 2, MMP-9 and HIF-1α (hypoxia-inducible factor 1- α) mRNA expression, tumor progression via regulation of HIF-1α signaling, which regulates cancer cell metabolism, proliferation, apoptosis and metastasis. Hypoxia also can induce basic fibroblast growth factor (FGF-2) to produce HIF-1α, resulting in vascular endothelium growth factor (VEGF) release and promotion of angiogenesis (Korc and Friesel 2009).

In the study conducted by Liu and Chang (2010), caffeine reduced invasion by modulating EMT and decreased mRNA and protein levels of MMP-2 and MMP-9 in leukemia U937 cells, via Ca2þ/ROS-mediated suppression of ERK/c-Fos pathway and activation of p38 MAPK/c-Jun pathway. Moreover, caffeine was able to block the migration of rat and human glioma cells and down-regulated the production of phosphorylated (p)-FAK and p-paxillin, proteins associated with focal adhesions development (Chen, Chou, et al. 2014b).

Maugeri et al. (Maugeri et al. 2018) studied caffeine action on inhibiting molecules associated to activating invasion and metastasis. This investigation evaluated the action of caffeine in glioblastoma multiforme (GBM) development by modulating hypoxic event. Since this type of cancer presents extensive hypoxic foci triggering, the study evaluated hypoxia-inducible factors (HIFs) expression, such as HIF-1α that has an important role in the induction of VEGF, which in turn is crucial to angiogenesis and cell migration. Also, this study evaluated the activation of phosphoinositide three kinase (PI3K)/Akt and mammalian

mitogen activated protein kinase/Erk kinase (MAPK/ERK) signaling pathways. The findings indicated that caffeine decreased HIF-1α and VEGF expression in GBM cells exposed to hypoxia. This result is achieved by inhibiting PI3K/Akt and MAPK/ERK signaling pathways both associated with HIFs modulation.

Hallmark 6: Inducing angiogenesis

Since cancer cells present a high index of proliferation, carcinogenic cells created some strategies to constant receive oxygen and nutrients as well as eliminate metabolic waste. This mechanism is based on stimulating a dense network of blood vessels, that contributes to the metastatic process. Some angiogenic activators are recruited, such as VEGF and TNF-α, and inhibitors of neovasculature are blocked, for example, thrombospondin, tissue inhibitor of metalloproteinase (TIMP). This process is mediated by healthy cells present in the tumor microenvironment and cancer cells (Hanahan and Weinberg 2011). Also, neovasculature counts on the presence of nitric oxide and many pro-inflammatory cytokines, such as IL-1β, IL-6, TNF-α and GM-CSF (Guruvayoorappan and Kuttan 2008).

Maugeri et al. (Maugeri et al. 2018) reported that caffeine presented antiangiogenic action by inhibiting HIF-1α and VEGF in glioma cells. In agreement with this study, Merighi et al. (Merighi et al. 2007) suggested that caffeine down-regulated HIF-1α and its downstream effector VEGF in colorectal cancer cells.

Guruvayoorappan et al. (Guruvayoorappan and Kuttan 2008) showed that (+)-catechin is able to promote angiogenesis inhibition by regulating NO and TNF-α production. An in vivo study was conducted using B16F-10 melanoma cell-induced capillary formation in C57BL/6 mice. The treatment of (+)-catechin significantly blocked the number of tumor-directed capillaries induced by injecting B16F-10 melanoma cells on C57BL/6 mice. These animals presented an increased production of proinflammatory cytokines, such as IL-1β, IL-6, TNF-α, GM-CSF and the direct endothelial cell proliferating agent, VEGF. On the other hand, animals that received (+)-catechin showed a significant reduction in these cytokines. Moreover, (+)-catechin-treated animals presented an increased production of TIMP-1. Also, (+)-catechin inhibited the microvessel outgrowth at nontoxic concentrations (5–25 μg/ml) in the rat aortic ring assay. This molecule was able to inhibit important events that are associated to angiogenesis development, such as proliferation, migration and tube formation of endothelial cells. In addition, (+)-catechin inhibited VEGF mRNA levels in B16F-10 melanoma cells.

Barcz et al. (Barcz et al. 1998) investigated the action of theobromine on angiogenic development and proangiogenic cytokines production of human ovarian cancer cells, that were acquired from ascitic fluid obtained during palliative punction with ultrasound control. Since adenosine is a remarkable molecule able to induce neovascularization, this study investigated whether theobromine, that is considered an adenosine receptor antagonist, could be able to inhibit angiogenic activity and proangiogenic cytokines production. The study was conducted on in vivo and in vitro models. To conduct the in vivo experiment, ovarian cancer cells obtained from 4 patients were preincubated with (or without) theobromine, after that cells were washed and injected intraperitoneally into inbred Balb/c mice. For the in vitro study, cells were treated with theobromine using cell culture method. The results indicated that theobromine presented a significant inhibition of angiogenic activity of ovarian cancer cells. Theobromine reduced vascular VEGF production. However, theobromine did not change basic bFGF and IL-8 production.

In summary, Figure 8 shows the actions of these bioactive molecules targeting into angiogenesis.

Hallmark 7: Resisting cell death

Programmed cell death, such as apoptosis, is an essential physiological process to prevent the development of cancer, as it prevents cells without guarantee of a healthy genome copy from continuing the division process and lasting in the body. This process involves some patterns, such as reduced cell size, fragmentation of the nucleus into particles the size of nucleosomes, while keeping membrane and cell contents intact (Elmore 2007).

Apoptotic process can be triggered by a succession of molecular biochemical events that culminate in the activation of caspases. The caspase family includes more than ten members and can be divided into two groups, initiators (for example, caspase-9 and caspase-8) and effectors (for example, caspase-3 and caspase-7). The apoptotic process is initiated by the activation phase, in which the caspases become catalytically active, and followed by an execution phase, where these enzymes act causing the cell to die. Initially, apoptosis occurs, mainly by two distinct pathways, however both converge to activate caspases, the extrinsic or cytoplasmic pathway, mediated by the receptors, and the intrinsic or mitochondrial pathway (Elmore 2007).

The extrinsic pathway is mediated by activating receptors, such as the TNF- α and CD95, which activate the initiating caspases and, conduct the cell directly to the effector phase. The intrinsic or mitochondrial pathway, on the other hand, results from the increased permeability of the mitochondria and the release of pro-apoptotic molecules into the cytoplasm, without the participation of death receptors. Anti-apoptotic molecules, such as Bcl-2 and pro-apoptotic molecules, such as Bax, are directly involved in this process. When the cell suffers an injury or stress, the levels of Bcl-2 decrease and in contrast the levels of Bax increase, this generates an increase in mitochondrial permeability and release of caspase-activating proteins, such as cytochrome C. In the last stage of apoptosis, the effector phase, initiating caspases activate effector caspases and, thus induce the proteolytic process of cellular constituents (D'Arcy 2019).

Some conditions of cellular stress, such as, exposure of cells to radiation or chemotherapeutic agents, can induce apoptosis via DNA damage, involving ATM and ATR and the tumor suppressor gene *p53*. When genotoxic stress is generated,

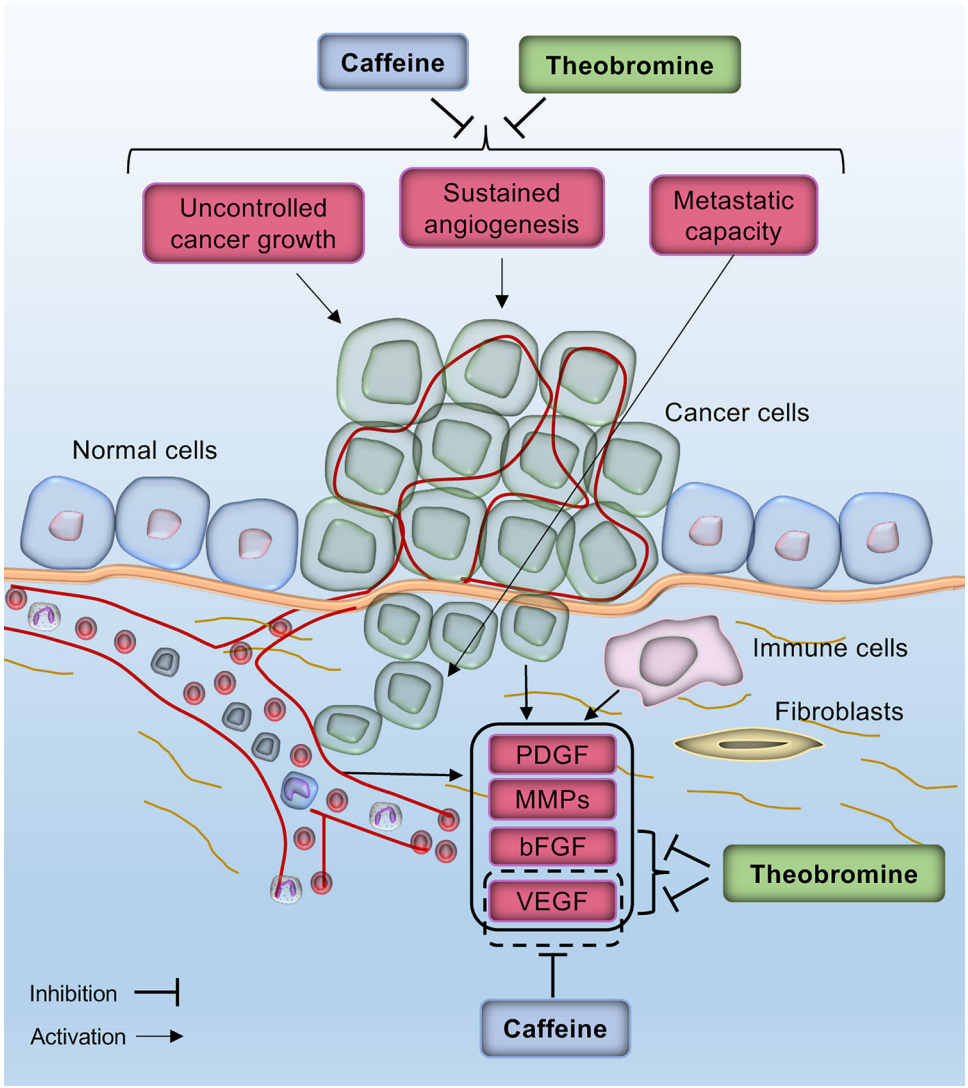


Figure 8. Caffeine and theobromine effects against invasion, metastasis and angiogenesis induction. Arrows indicate increased expression or activation. T bars indicate inhibition or reduced activity.

there is an accumulation of p53, generated by ATM and ATR action, that promotes cell cycle arrest in the G1 phase, in order to repair DNA injuries. If the DNA is not restored, p53 triggers cell death by apoptosis, thus ensuring that copies of cells with mutated DNA do not remain in the tissue, thereby preventing the development of tumor processes (Jeggo, Pearl, and Carr 2016). However, *p53* is frequently mutated or absent in most cancer cells, corroborating to cancer development (Vijayakumaran et al. 2015).

Manikandan et al. (Manikandan et al. 2012) investigated the anticancer activity of (+)-catechin against human colon adenocarcinoma HCT 15, HCT 116, and human larynx carcinoma Hep G-2 cell lines. The results indicated that (+)-catechin decreased cell proliferation promoting nuclear fragmentation as well as condensation, and DNA fragmentation related to the presence of apoptosis. Therefore, cell treated with (+)-catechin increased chromatin fluorescence, condensed nuclear morphology, and the presence of apoptotic bodies in the cancer cells.

Cadoná et al. (Cadoná et al. 2016) reported that caffeine and (+)-catechin presented cytotoxicity in colorectal cancer cells (HT-29) since they were able to stimulate apoptosis. Caffeine up-regulated caspase 3 and (+)-catechin was able to up-regulate caspases 8 and 3. In addition, in this same study theobromine presented antiproliferative activity against colorectal cancer cells (HT-29) by modulating gene expression associated with growth pathway. Bax/Bcl-2 ratio was strongly up-regulated in cells exposed to theobromine significantly decreasing cell proliferation.

Figure 9 shows the findings described in this topic.

Hallmark 8: Deregulating cellular energetics

The target protein of rapamycin in mammals (mTOR) is a central molecule in the control of cell growth and proliferation, metabolism and angiogenesis. The functions of the mTOR pathway involve two protein complexes, mTORC1 and mTORC2. The mTORC1 complex responds positively to the presence of amino acids, oxygen, energy levels. Both mTORC1 and mTORC2 are active due to growth stimulus, as for example, insulin (Laplante and Sabatini 2012).

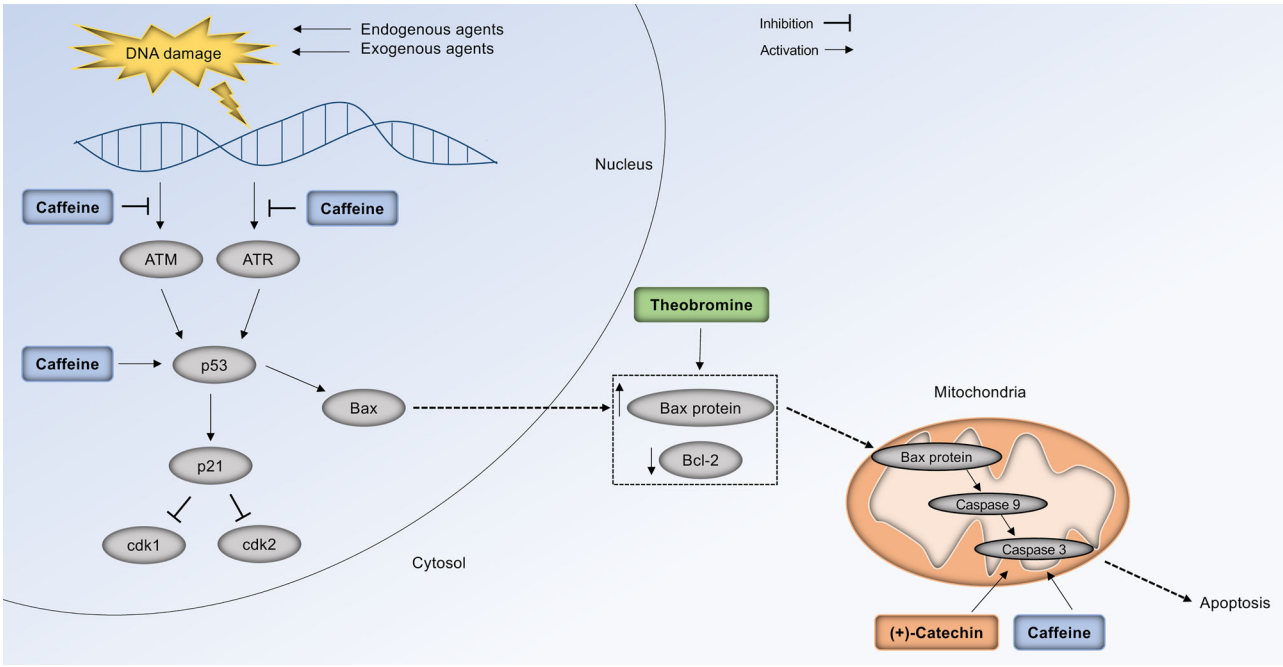


Figure 9. Molecular targets of caffeine, (+)-catechin and theobromine in apoptosis pathway and repair DNA damage via ATM and ATR pathway. Arrows indicate increased expression or activation. T bars indicate inhibition or reduced activity.

Thus, when activated, mTORC1 allows the synthesis of macromolecules, such as proteins and lipids, promotes the progression of the cell cycle, as well as the growth and metabolism of the cell. It also blocks autophagy processes and lysosomal synthesis, which are essential for eliminating senescent cells and organelles. On the other hand, mTORC2 supports the metabolism, organization of the cellular cytoskeleton and cell survival. These processes are regulated through phosphorylation and the consequent activation of various protein molecules. mTORC1 controls the phosphorylation of its substrates, S6K1 and 4E-BP1, while mTORC2 regulates the phosphorylation of different AGC kinases, including AKT, serum/glucocorticoid regulated kinase 1 (SGK1) and protein kinase C alpha (PKC-α) (Ma and Blenis 2009).

The mTOR signaling pathway is frequently deregulated in many types of cancers. Thus, studies have shown the important role of mTOR for the development and progression of cancer. Often, mTORC1 and mTORC2 are mutated and overexpressed in cancerous processes, which stimulates several essential pathways for the growth of cancer cells, proliferation and survival. Additionally, the loss of *p53* activity, which affects most cancers, promotes the activation of mTORC1 (Guertin and Sabatini 2009). Therefore, the inhibition of this route is of interest for the development of pharmacological therapies (Beck 2015; Saiki et al. 2011).

In addition, *PTEN* (tensin homologous phosphatase) is considered a tumor suppressor gene that is often deregulated in various types of cancers, such as breast and prostate cancer. PTEN acts by negatively regulating the PI3K/PKB/Akt signaling pathways, which are part of the mTOR2 pathway. Also, an over-stimulation of AKT blocks the mTOR1 inhibitor (TSC1), over-regulating also mTOR1. As a result of the absence of inhibition of this pathway by mutation in PTEN, there is an overactivation of mTOR2 signaling, which stimulates cell proliferation and survival. Thus, studies that reveal new agents able to upregulate *PTEN* could be used as a therapy against cancer (Martelli et al. 2011).

Cadoná et al. (Cadoná et al. 2017) reported that caffeine was cytotoxic against breast cancer cells line (MCF-7) by inhibiting mTOR pathway. Caffeine modulated the mTORC1 and mTORC2 pathway in MCF-7 by inhibiting their substrates, p-SK6 and p-AKT, respectively. Moreover, Maugeri et al. (2018) reported that caffeine presented anticancer action in glioblastoma multiforme (GBM) cells line. Caffeine was able to decrease cellular proliferation by inhibiting of PI3K/Akt pathway.

In addition, a previous investigation showed evidence that caffeine inactivates PI3K and AKT and activates PTEN in of human osteosarcoma cells (MG63) fibrosarcoma cells (HT1080) (Miwa et al. 2012). Therefore, this molecule plays a crucial role in modulating metabolic pathways in cancer cells that unleash apoptosis. Besides, a study reported that caffeine promoted PTEN activation and Akt inactivation, inhibiting human osteosarcoma cells (MG63) and fibrosarcoma cells (HT1080) (Miwa et al. 2011)).

Also, Rosendahl et al. (Rosendahl et al. 2015) reported that caffeine reduced the insulin-like growth factor-I receptor (IGF-IR) and pAkt levels in both ER+ (MCF-7) and ER- (MDAMB-231) cells, causing cell cycle arrest and cell death.

Moreover, Sugimoto et al. (Sugimoto et al. 2014) reported that theobromine presented anti-cancer activity against malignant glioma cell line (U87-MG). The results indicated that theobromine reduced p44/42 extracellular signal-regulated kinase action and the Akt/mammalian target of rapamycin kinase and nuclear factor-kappa B signal pathways. This action mechanism generated an important antiproliferative role against U87-MG cells.

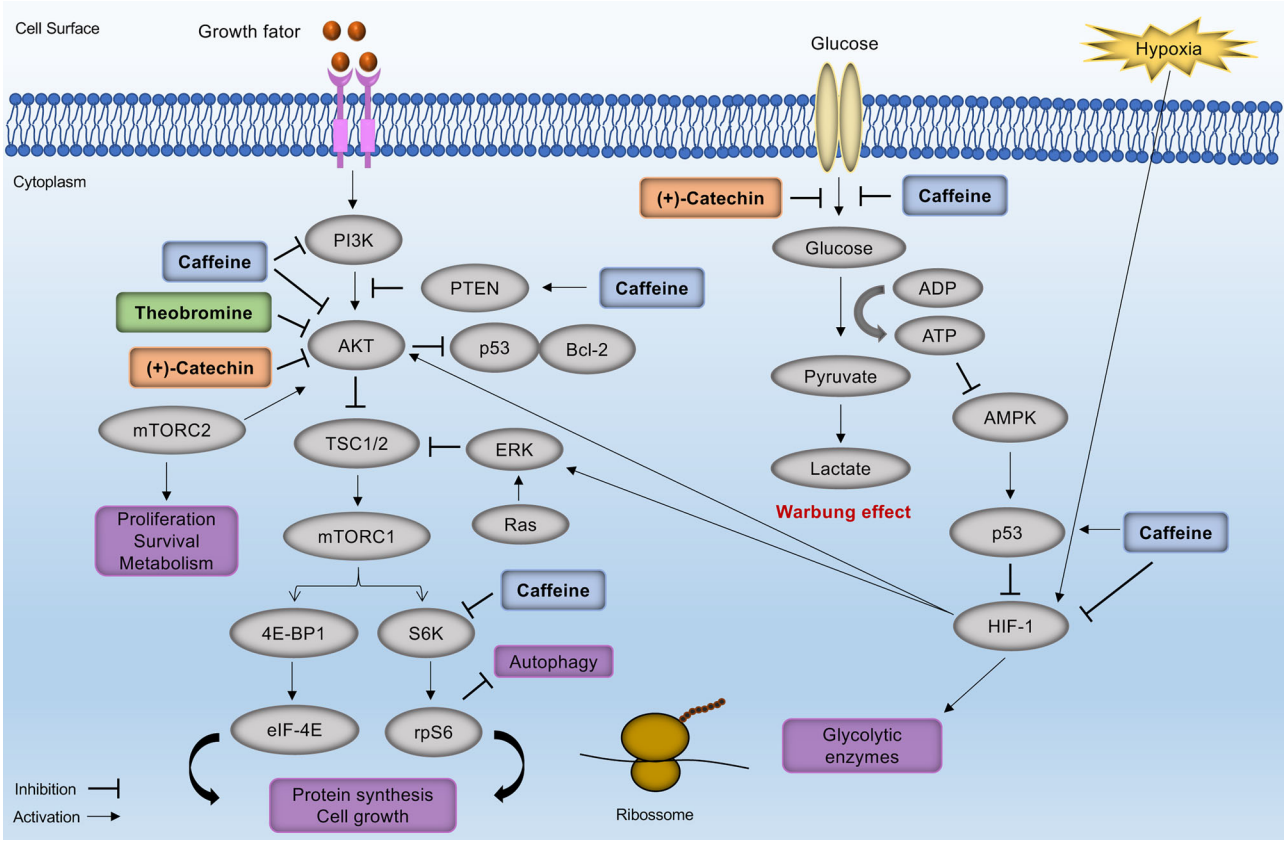


Figure 10. Caffeine, (+)-catechin and theobromine action in cellular energetics pathways. Arrows indicate increased expression or activation. T bars indicate inhibition or reduced activity.

Furthermore, a study by Sun et al. (Sun et al. 2020) reported the anti-cancer effect of (+)-catechin targeting into modulate cellular energetics pathways. The results suggested that (+)-catechin was able to inhibit the expressions of cyclin E1 and phosphorylation of p–AKT in a dose-dependent manner in non-small cell lung cancer A549 cells. This mechanism was crucial to block cancer cells growth.

Another very singular feature of cancer cells that should be emphasized in this hallmark is the ability of cancer cells to prefer using glycolysis, even in situations of high levels of oxygen. This event is called anaerobic glycolysis or Warburg effect to differentiate from the normal glycolysis, performed by healthy cells. HIF-1 plays an important role in this pathway, as well as adenosine 5′-monophosphate-(AMP-) activated protein kinase (AMPK), PI3K; AKT and ERK are essential to activate glucose metabolism. HIF-1 upregulates the glucose transporters, mainly glucose transporter 1 (GLUT1) and GLUT4, as well as it increases the expression of hexokinase (HK), pyruvate kinase (PK), and lactate dehydrogenase (LDH-A), that are enzymes involved with glycolysis pathway. On the other hand, p53 and von Hippel-Lindau (VHL), two important tumor suppressors, block this process (Gao and Chen 2015).

Therefore, investigations using natural products have been performed to reveal new glycolysis inhibitors to control cancer cells growth and metastasis. A previous study reported that (+)-catechin was able to inhibit glucose uptake in human myeloid leukemia cell line U937 cells (Park 1999). Moreover, Lin et al. (2014) (Lin et al. 2014) investigated the effect of caffeine in genes that control glucose metabolism in HeLa cells. They suggested that caffeine modulates some p53 isoforms, that are associated with p53 activity. This bioactive molecule decreased $p53_a$ expression and promoted the expression of $p53_\beta$, which contains an alternatively spliced p53 C-terminus, by the alternative splicing of the target genes of serine/arginine-rich splicing factor 3 (SRSF3). Besides, caffeine also stimulated the alternative splicing of other SRSF3 target genes, such as GLUT1, HIF-1_a, and HIF-2_a, suppressing their expression.

A summary of the findings related to actions of bioactive molecules focus on cellular metabolism modulation are shown in Figure 10.

Enabling characteristic 1: Tumor-promoting inflammation

Some previous investigations have reported that the microenvironment of tumors is also composed of inflammatory cells. This ensures cancer cells survive and progress, since inflammatory cells can provide to cancer cells cytokines and proteases. These inflammatory molecules allow cancer cells to rapid proliferate, survive and metastasize. ROS production are present in the tumor environment promoting cancer cell proliferation and inflammation pathways, allowing angiogenesis, metastasis, and survival (Aggarwal et al. 2019). In this sense, ROS production activates a variety of cellular pathways that a measure of cell stress. However, cancer cells,

which are set with a high concentration of ROS, are sensitive to an additional generation of these molecules, since these cells have reduced and unregulated antioxidant defense mechanisms. Therefore, an increase in the production of these molecules can trigger apoptosis (Philion et al. 2017). Also, nitric oxide (NO) and several pro-inflammatory cytokines, for example IL-1β, IL-6, TNF-α and GM-CSF, can promote cancer survival and contribute to metastasis process (Guruvayoorappan and Kuttan 2008).

A previous study conducted by Wang et al. (Wang et al. 2019) investigated the anticancer action mechanism of caffeine via determination of ROS. They reported that caffeine presented anti-cancer activity in hepatocellular carcinoma cells, as well as improved the chemotherapy response of 5-fluorouracil (5-FU), in vitro and in vivo. To perform the in vitro experiments, hepatocellular carcinoma cell lines (SMMC-7721 and Hep3B) were used, while, in vivo assays were conducted using male BALB/c mice, that received SMMC-7721 subcutaneously injected into the dorsal region to establish a subcutaneous tumor formation model in nude mice. The results suggested that caffeine combined with 5-FU decreased cell proliferation by modulating proteins involved in apoptosis pathway. For instance, protein expression level of cleaved PARP was up-regulated while the protein levels of Bcl-2 and Bcl-xL were down-regulated. Moreover, ROS levels were increased in the 1 mM caffeine and 25 μM 5-FU combination group when compared to the control or single drug group.

Guruvayoorappan et al. (Guruvayoorappan and Kuttan 2008) reported the anti-cancer activity of (+)-catechin by analyzing proinflammatory cytokines, that are involved in cancer growth and invasion. In vivo experiments were performed, using B16F-10 melanoma injected in C57BL/6 mice. The results showed that the administration of (+)-catechin did not change the levels of IL-1β levels in the initial periods (24 h). However, the treatment decreased the IL-1β level close to normal by day 9 after angiogenesis induction. In addition, (+)-catechin significantly reduced the levels of IL-6 in the serum of the animals on day 1 and day 9 after tumor inoculation. Also, granulocyte-macrophage colony-stimulating factor (GM-CSF) was reduced by the (+)-catechin treatment on day 1 and day 9.

Enabling characteristic 2: Targeting genome instability and mutation

Frequently, cells suffer DNA injuries of endogenous and exogenous factors. To repair genomic damage, cells present a sophisticated system that avoid them to keep mutated DNA. Ataxia telangiectasia mutated (ATM) and RAD3-related (ATR) kinases are the key to mediate this pathway. These molecules cause cell cycle arrest and induce DNA repair. In this sense, investigations have been performed to look for anti-cancer agents via DNA damage response inhibition, mainly involving ATM and ATR blockage, since many types of cancer present treatment resistance by increasing DNA repair pathway. Also, some cancers have certain components from the DNA damage response altered, therefore rendering them highly dependent on the rest of this pathway for remaining alive (Weber and Ryan 2015).

Sarkaria et al. (Sarkaria et al. 1999) investigated whether caffeine present anti-cancer activity by analyzing ATR and ATM pathways in A549 lung carcinoma. A549 cells were exposed to γ - and UV radiation to activate DNA damage checkpoints and after cells were treated with caffeine. The results indicated that caffeine caused disruption of multiple DNA damage-responsive cell cycle checkpoints by inhibiting ATR and ATM, decreasing cancer cell growth. Corroborating with this study, Tsabar et al. (Tsabar et al. 2015) investigated the anti-cancer activity of caffeine in cervical cancer cells line (HeLa) by evaluating ATR and ATM.

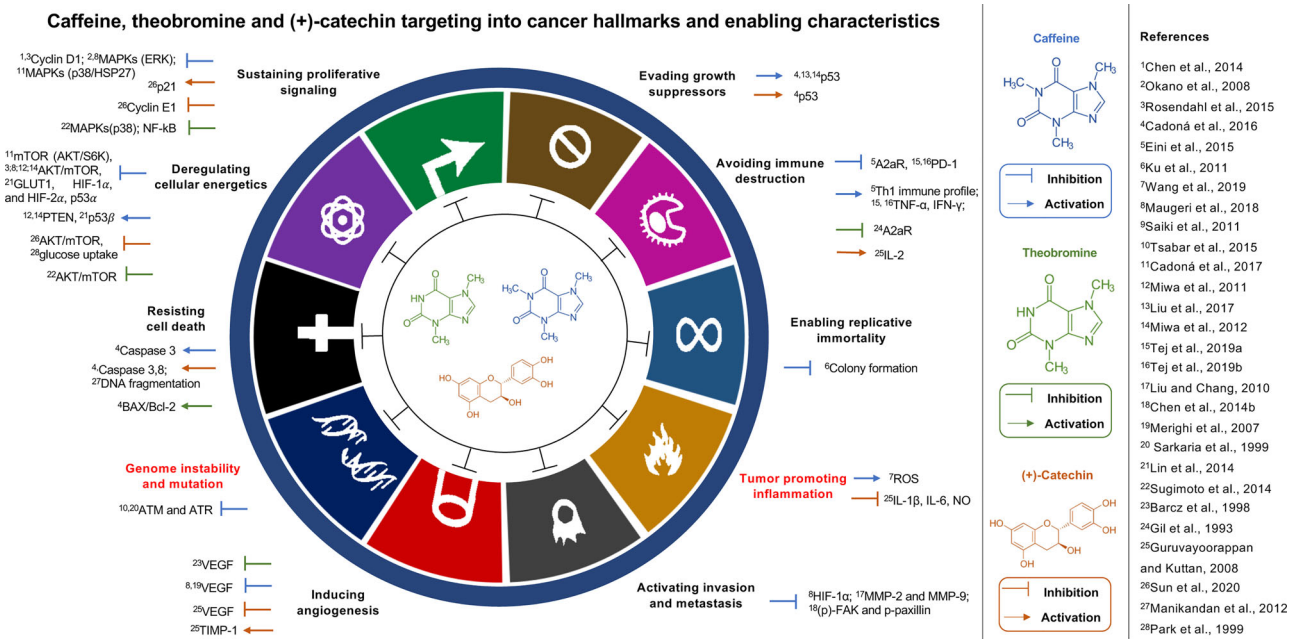


Figure 11. Caffeine, (+)-catechin and theobromine targeting into cancer hallmarks (in black) and enabling characteristics (in red).

The results indicated that caffeine was able to inhibit ATR and ATM promoting cancer cells death. 1.

Conclusion

In conclusion, this review summarized that caffeine, (+)-catechin and theobromine were able to interact with several cancer hallmarks. A graphical depiction of the conclusions of this study can be seen in Figure 11. Therefore, since these bioactive molecules present remarkable anticancer activity by modulating different cancer hallmarks, more studies should be conducted to develop new therapies based on these natural products to reveal new therapeutic agents that could be used for cancer treatment.

Declaration of interest statement

The authors declare no conflict of interest.

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