The chemopreventive activity of apple against carcinogenesis: antioxidant activity and cell cycle control

Apples and their derivatives are rich in phytochemicals, including flavonoids (catechins, flavonols, quercetin) and phenolic acids (quercetin glycosides, catechin, epicatechin, procyanidins), vitamins, and fibers, that confer an important antioxidant property. Chemoprevention is defined by the use of natural or synthetic agents to interfere with the progression, reverse, or inhibit carcinogenesis, thereby reducing the risk of developing clinically invasive disease. The aim of this article is to present data generated from the use of apples as a chemopreventive agent in carcinogenesis using in-vivo and in-vitro test systems. Apple and its bioactive compounds can exert chemopreventive properties as a result of antioxidant activity and cell cycle control. However, future focus of research on apple such as identifying the specific phytochemical responsible for the anticarcinogenic effect, timing of consuming, and adequate amount of apples to achieve the best preventive effect using human large randomized-controlled trials is needed. Furthermore, animal studies are also relevant for better understanding the role of this fruit in human health as well as modulation of degenerative diseases such as cancer. Therefore, this area warrants further investigation as a new way of thinking, which would apply not only to apples but also to other fruit used as promising therapeutic agents against human diseases.

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Introduction
In the last few years, some evidence has been found that healthy eating and regular consumption of functional foods prevents the manifestation of certain chronic diseases such as cardiovascular disorders, diabetes, Alzheimer’s disease, and cancer (Miura et al., 2008; Poulsen et al., 2011; Liu, 2013). In fact, some studies have shown that one-third of all cancer deaths could be prevented by diet (Hyson, 2011), and these benefits are often attributed to the high phytochemical content and antioxidant power that are present in some functional foods (Serra et al., 2010).

Mechanisms of action of phytochemicals in cancer chemoprevention are diverse such as the antioxidant activity of free radical scavenging (Liu et al., 2005), regulation of gene expression in cell proliferation, cell differentiation, oncogenes, and tumor suppressor genes; induction of cell-cycle arrest and apoptosis; modulation of enzyme activities in detoxification, oxidation, and reduction; stimulation of the immune system; regulation of hormone metabolism; and antibacterial and antiviral effects (Liu et al., 2004). Epidemiological, clinical, and in-vitro studies have reported a plethora of biological effects related to the phenolic compounds present in the human diet, such as antioxidant, anti-inflammatory, antimicrobial, and anticarcinogenic actions (Gusman et al., 2001; Delmas et al., 2005).

Apples and their derivatives have been investigated widely because of their phytoconstituents (Hyson, 2011; Chirumbolo, 2012). Apples are rich in phytochemicals, including flavonoids (catechins, flavonols, quercetin) and phenolic acids (quercetin glycosides, catechin, epicatechin, procyanidins), vitamins, and fibers (Gallus et al., 2005; Setorki et al., 2009; Wang et al., 2009; Serra et al., 2010; Ravn-Haren et al., 2013), which confer an important antioxidant property (Walia et al., 2012). All these compounds are distributed in the peel, core, and pulp, and they are absorbed by the body, validating their beneficial effects on health. Although some phenolic compounds are degraded by microorganisms, they are still adsorbed and act in cell signaling, cell growth, proliferation, and apoptosis (Gerhauser, 2008). Notably, some authors have provided evidence that the consumption of apples as well as apple extract is associated with a decreased risk of development of degenerative diseases such as cancer, diabetes, cardiovas-
cicular diseases, and those related to the formation of reactive oxygen species (Soyalan et al., 2011).

In particular, some of these apple compounds are already being used in experimental cancer treatment (Palermo et al., 2012) such as pectin, a polysaccharide fiber that can block proliferation and induces cellular apoptosis (Gerhauser, 2005; Niture and Refai, 2013). The major role of this (compound) in cancer is protection against oxidation, which can lead to DNA damage (Boyer and Liu, 2004). Studies have reported anticancer properties of bioactive compounds from apple because of its antioxidant activity (Liu et al., 2005), modulation of gene expression, induction of apoptosis, decrease in platelet aggregation, increase in blood vessels’ dilatation, modulation of intercellular signaling, modulation of enzyme activities associated with carcinogen activation, and detoxification and/or chelation of transition metals, such as iron (De Moura et al., 2013).

Apple is a food that is consumed frequently by humans. Compared with other lifestyle factors, diet is easy to modify. Review of the anticarcinogenic properties of apples is important to provide the scientific grounds to support future studies, such as large-scale human studies. The aim of this article is to present data generated from the use of apple as a chemopreventive agent in carcinogenesis using in-vitro and in-vivo test systems.

Chemoprevention studies using apples
Taking into consideration the multistep carcinogenesis assay as a result of initiation-promotion steps (Berenblum and Armuth, 1981; Heidelberger et al., 1983), many potential cancer-protective agents may be categorized as blocking agents that can stop the initiation stage or that arrest or reverse the promotion and progression of cancer, affecting or perturbing crucial factors that control cell proliferation, differentiation, senescence, or apoptosis (Chen and Kong, 2005). These may occur through chemoprotective effects from apples; thus, the anticarcinogenic function of these compounds might be attributed to a combination of their cytoprotective effect on normal cells and their cytotoxic effect on neoplastic and/or neoplastic cells (Aggarwal and Shishodia, 2006).

Apples are ranked as the second for the total phenolic content and have the second highest total antioxidant activity among 21 fruit and vegetables consumed commonly in the USA (Chu et al., 2002; Sun et al., 2002; Wolfe et al., 2003; Yoon and Liu, 2007).

Apples and their compounds act as an antioxidant agent, improving the production of oxidative stress enzymes (Soyalan et al., 2011). Previous studies have reported that apple polyphenols inhibit inflammatory gene expression and are potent antioxidants in vitro (Schafer et al., 2006a, 2006b; Bellion et al., 2008; Jung et al., 2009).

According to Eberhardt et al. (2000), vitamin C in apples only contributed less than 0.4% of the total antioxidant activity, suggesting that the complex mixture of phytochemicals in fruit and vegetables provides health benefits, including antioxidant and antiproliferative processes. Avci et al. (2007) carried out a study in Turkey and evaluated antioxidant enzymes in participants who ate fresh apples (2 g/kg) for a month. The authors noted an increase in the concentration of enzymes such as SOD and glutathione peroxidase activity in erythrocytes and plasma, suggesting that the apple can reduce oxidation.

Thompson et al. (2009) compared the chemical properties and antioxidants in four common apple varieties in inhibiting the growth of cancer cells in vitro. According to these authors, all the extracts tested could inhibit the proliferation of tumor cells. However, the same was not observed in an in-vivo study; a study carried out by Wang et al. (2009) to investigate the association between the ingestion of selected flavonoids and flavonoid-rich food and the risk of cancer using in-vivo and in-vitro assays. Flavonoids could not confer protective effects on the risk of cancer when administered at the same levels of human consumption.

The antiproliferative capacity of six varieties of apples and other fruit was compared against four cell lines: human lung cancer cells (A549), human breast cancer cells (MCF-7), human hepatoma cells (HepG2), and human colon cancer cells (HT-29). All samples showed different growth-inhibition activities toward all cell lines. According to Lapidot et al. (2002), the antiproliferative effect of apple extract is not because of the high concentration of antioxidant compounds, but the indirect formation of hydrogen peroxide (H₂O₂) through the interaction between phenolic compounds and cell culture media components. This leads to oxidative stress and activates mechanisms that can promote or decrease apoptosis and increase or repress proliferation, and enzymes such as peroxidases, lipoxygenases, and cyclooxygenases. However, Liu and Sun (2003); Veeriah et al. (2006) did not obtain the same results, but the authors explained that this might be because of the different methods used to measure the H₂O₂ content; Janzowski et al., in a personal communication to Gerhauser (2008), reported that H₂O₂ formation only occurred in bicarbonate-buffered solutions, suggesting that this mechanism is not relevant to apple antiproliferative assays.

Previous studies have shown that apple induces apoptosis in various cancer cell lines (Liu and Sun, 2003; Gossé et al., 2005; Kern et al., 2005; Zheng et al., 2013). Miura et al. (2008) studied whether apple polyphenols, apple condensed tannins, can trigger apoptosis in tumor cells in an in-vitro assay. They concluded that apple condensed tannins can induce apoptosis in cancer cells by activating caspase-3 through mitochondria damage-related caspase-9. The same result was obtained by Zheng et al. (2013), who observed an increase in the apoptosis levels, by caspase-3
analysis, in the metastatic cell line (ACC-M) treated with apple polyphenols in different concentrations at both mRNA and protein levels. They showed that, after treatment with 150 or 250 µg/ml of apple juice, cultured ACC-M cells showed morphologic changes in the appearance of typical morphological apoptosis such as rough cell faces, condensed nuclei, and presence of apoptotic bodies.

The apoptotic activity of polysaccharides of apples (APs) was also investigated in human colorectal cancer cells by Zhang et al. (2012). At concentrations of 0.1 and 1 mg/ml, APs could induce apoptosis in HT-29 and SW620 cells, as well as increase the expression of Bax, nuclear p65, and cytoplasmic IκBα, and decrease Bcl-2, Bcl-xl and cytoplasmic IκBα expression. Given these results, the authors concluded that APs induce apoptosis through activation of the NF-κB pathway.

According to Liu et al. (2009), the inhibition of cell proliferation and induction of apoptosis in mammary cancer may be regulated through the downregulation of cyclin D1 and Bcl-2 expression as well as the upregulation of Bax expression. Yoon and Liu (2007) assessed whether apple extract can activate NF-κB by TNF-α in human breast cancer cells (MCF-7). Their results showed that apple extracts (40 mg/ml) inhibited cell proliferation without cytotoxicity and blocked NF-κB activation by inhibiting proteasome activity.

Fini et al. (2007) tested the anticancer properties of apple extract in colorectal cancer in vitro and showed that apple extract inhibited cell viability in a time-dependent and concentration-dependent manner. These authors showed that apple extracts lead to the reactivation of silenced tumor suppression genes through inhibition of DNA methyltransferases DNMT-1, which contributes toward DNA promoter methylation cancer, and DNMT-3b, which cooperates to maintain DNA methylation status. Kern et al. (2005) also found that the polyphenolic-rich extract of a consumer-relevant apple juice extract potently inhibited the growth of human colon carcinoma cells (HT-29) in vitro and effectively blocked the activity of isolated epidermal growth factor receptor, an effect that might be interesting with respect to chemoprevention.

Barth et al. (2005) induced colonic damage with 1,2-dimethylhydrazine in rats and treated them with two different preparations of apple juice; they showed a decrease in important markers such as DNA damage and hyperproliferation, and there was a decrease in the number of large aberrant crypt foci in the distal colon. The aim of this study was to evaluate the effects of apple in relation to colon cancer; it was observed that 7 weeks of daily consumption protected the mucosa against the carcinogenic agent. In follow-up studies, the same authors examined the effect of isolated fractions on the above markers and concluded that apple juice was more effective than individual components of juice, including polyphenolic-rich extracts (Barth et al., 2007). Femia et al. (2012) investigated whether a high-fat diet (23% w/w corn oil) added with apples of Marie Ménard variety (7.6% w/w lyophilized) modulated 1,2-dimethylhydrazine-induced rat colon carcinogenesis. The supplementation of apple reduced the number of precancerous lesions as a result of a strong chemoprotective effect by increasing apoptosis of neoplastic cells (Femia et al., 2012).

Poulsen et al. (2011) induced colon cancer in rats and evaluated the possible chemoprotective effect promoted by daily intake of fresh apples. The results showed that apple reduced the total number of aberrant crypt foci. In fact, after 6 weeks, a 50% reduction in the total number of hyperproliferative crypts was also observed in rats injected with the chemical carcinogen azoxymethane, known to cause colon cancer, during 2 weeks and after treatment with apple procyanidins in their drinking water. This indicates that apple procyanidins administered in the drinking fluid inhibited the promotion/progression phases of colon carcinogenesis in addition to their potential protective effects as antioxidants in cancer initiation (Gossé et al., 2005).

Gossé et al. (2005) observed that treatment with apple procyanidin led to the accumulation of cells at the G2/M cell cycle phase. This was accompanied by a simultaneous decrease in cells associated with the G0/G1 phase and the procyanidin fraction from apple inhibited the growth of metastatic cells.

**Conclusion**

In this article, we have reported recent studies focusing on the chemopreventive properties exerted by apples against carcinogenesis using in-vitro and in-vivo test systems. Although these data have shown important mechanisms of action, much remains to be examined. Future focus of research on apples such as identification of the specific phytochemical responsible for the anticarcinogenic effect, timing of consumption, and adequate amount of apples to achieve the best preventive effect using human large randomized-controlled trials is needed. Furthermore, animal studies are also relevant for better understanding the role of this fruit in human health as well as modulation of degenerative diseases such as cancer. Therefore, this area warrants further investigation as a new way of thinking, which would apply not only to apples but also to other fruit used as promising therapeutic agents against human diseases.

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**Conflicts of interest**

There are no conflicts of interest.
References
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