Towards a Global Assessment of the Anticancer Properties of Fruits and Vegetables: the Montreal Anticancer Nutrinome Project

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Abstract

Epidemiological studies has consistently linked abundant consumption of fruits and vegetables to a reduction of the risk of developing several cancers. The mechanisms responsible for this chemopreventive effect still remain largely unknown but is likely related to the presence of phytochemicals associated with fruits and vegetables. In this paper, we summarize the currently available data suggesting that a number of phytochemicals interfere with several key steps that are essential for tumor progression, thereby maintaining tumors in a latent and inoffensive state. The identification of fruits and vegetables containing phytochemicals with the highest anticancer properties as well as the characterisation of the mechanisms by which these foods elicit their anticancer actions thus represent an essential step for the development and implantation of dietary-based chemopreventive approaches.

The Impact of Diet on the Development of Cancer

Dietary factors may account for approximatively 35% of cancer death, similar to the impact of smoking (Doll and Peto, 1981). Such a close relationship between diet and cancer is well illustrated by the large variations in rates of specific cancers among countries as well as by the spectacular changes observed in the cancer incidence rates in migrating populations (Willett, 2000). Among specific aspects of the diet that are the most closely associated with cancer, a large number of ecological, case-control and cohort epidemiological studies have consistently associated increased consumption of fruits and vegetables with a decreased risk for a wide variety of cancer, particularly those of epithelial origin (Gescher et al., 1998). In these studies, people consuming the least fruit and vegetables are about two-fold more susceptible to develop some cancers than those having the highest consumption of these foods. These studies thus suggest that fruits and vegetables represent an essential source of molecules with chemopreventive properties, an hypothesis strengthened by many experimental data obtained using cellular and animal models in which molecules isolated from several food sources were found to induce tumor cell death as well as to reverse the development of several cancers (Surh, 2003; Dorai and Aggarwal, 2004).

Fruits and Vegetables are an Important Source of Anticancer Agents

In spite of considerable evidence linking the consumption of fruits and vegetables to a reduction of cancer risk, the identification of the biologically active molecules that are responsible for the chemopreventive properties of these foods is still a matter of intensive investigation.

Research carried out during the last years has shown that fruits and vegetables are rich sources of phytochemicals, non-nutritive molecules that play essential roles in various aspects of plant physiology, especially in their defense mechanisms against insects and various microorganisms. A wide variety of phytochemicals have been described to date and are classified by protective function, physical characteristics and chemical characteristics. The three major classes of phytochemicals are the polyphenols (flavonoids, isoflavones, anthocyanins, catechins, etc.), terpenes (including the subclasses carotenoids and limonoids) and the thiols (including the subclasses indoles, dithiolthiones

and isothiocyanates).These molecules are responsible for most of the color, odor and astringency of fruits and vegetables, and are present in significant amounts in plant-based foods : a daily intake of a mixture of fruits, vegetables and drinks such as green tea and red wine contains about 1 to 2 g of these phytochemicals, corresponding to the ingestion of about 5,000 to 10,000 different compounds (Ames et al., 1990).

One of the best characterized biological activity of phytochemicals is their antioxidant properties (Prior, 2003). For example, a medium-sized apple, which contains 10 mg of vitamin C has an antioxidant potential similar to that of 2350 mg of this vitamin (Eberhardt et al., 1990), a property mostly due to its high content in polyphenols such as flavonoids and procyanidins (Lee et al., 2003). However, although the antioxidant properties of phytochemicals have recently received enormous attention, there is concluding evidence that these compounds possess several additional anticancer properties that play crucial roles in the chemopreventive properties of fruits and vegetables (Gescher et al., 1998; Surh, 2003).

How do Fruits and Vegetables Prevent Cancer?

The recognition of fruits and vegetables as a source of anticancer molecules has led to considerable interest in the identification and characterization of the mechanisms by which naturally occurring phytochemicals found in the diet are capable of inhibiting, retarding or reversing carcinogenesis (Gescher et al., 1998). To date, at least four major mechanisms have been proposed to account for the chemopreventive properties of phytochemicals:

1. Stimulation of the Host's Defense Mechanisms against DNA Damaging Events. A number of factors can induce damage to DNA and initiate cancer. Free radicals, environmental or diet-associated chemicals, UV irradiation or some viruses all have the capacity to cause significant damage to the cells that may ultimately lead to cancer (Ames et al., 1995). Most, if not all environmental carcinogens are metabolized once they enter the body by the so-called Phase I metabolism, a physiological reaction primarily catalyzed by the cytochrome P450 enzymes (Nelson et al., 1993). However, this reaction often converts procarcinogens into highly reactive chemical intermediates that can bind and alter the function of key cellular macromolecules such as DNA. A second group of enzymes, known as Phase II enzymes, conjugate these recative intermediates with a number of endogenous factors, resulting in the production of water-soluble products that can be excreted by the body (Conney, 2003).

There is considerable evidence that several chemopreventive phytochemicals elicit their anticancer effects by modulating these enzymatic systems, either by reducing their carcinogenic potential (through the inhibition of the Phase I enzymes) or by increasing the excretion of the carcinogens, through increase of Phase II enzymes activity (Conney, 2003). The most well-documented example of such a mechanism of action is the remarkable anticarcinogenic activity of isothiocyanates, compounds that are found in high amounts in cruciferous vegetables. Isothiocyanates inhibit tumorigenesis induced by a wide variety of chemical carcinogens, this effect being related to their reduction of genetic damage as a result of the inhibition of Phase I enzymes and activation of Phase II enzymes (Hecht, 1999). A number of phytochemicals that modulate the host's defense mechanism against DNA-damaging molecules are also found in several other fruits and vegetables, including garlic and its related members of the *Allium* family, as well as citrus fruits. The presence of these biologically molecules causing the reduction of the oncogenic potential represents an efficient first-line defense against cancer that certainly contribute to the chemopreventive properties of fruits and vegetables.

2. Cytoxicity against Tumor Cells. In vitro experimental systems using cells isolated from various human tumors are being widely used to study the anticancer properties of dietary-derived phytochemicals. A large number of molecules have been shown to cause significant damage to cancer cells, leading to growth arrest and, in several cases, to the induction of apoptosis. Of particular interest is the strong proapoptotic activity of several isothiocyanates from cruciferous vegetables, such as phenethyl isothiocyanate (PEITC)

(Thornalley, 2002), as well as the potent cytotoxic effect of curcumin against several tumor cell lines (Karunagaran, 2005). How these cytotoxic properties contribute to the chemopreventive effects of these phytochemicals remains unclear but could play an essential role by preventing the growth of cells that have already acquire an initiated phenotype (precancerous cells).

3. Antiangiogenic Properties. Tumor angiogenesis, the process by which tumor cells stimulate the formation of a new blood vessel network that is necessary for their growth and progression, plays an essential role in tumor growth (Folkman, 2003). As a consequence, inhibition of angiogenesis has been proposed to represent an effective mean to interfere with tumor progression, leading to extensive efforts aimed at the identification of antiangiogenic molecules (Nyberg et al., 2005). Interestingly, many phytochemicals have recently been shown to possess strong antiangiogenic activities (Tosetti et al., 2002). For example, an abundant polyphenol found in green tea, epigallocatechin gallate (EGCG), was found to potently inhibit a crucial receptor involved in angiogenesis (VEGFR-2), this inhibitory effect occurring at concentrations readily achievable by the consumption of moderate amounts of green tea (Lamy et al., 2002). This inhibitory effect is not restricted to green tea catachins since a potent VEGFR inhibitory activity was also recently identified for delphidin, an abundant blueberry anthocyanidine (Lamy et al., 2005). In a similar manner, ellagic acid, a phenolic acid found in high quantities in some fruits, such as raspberries and strawberries, also interfere with VEGFR-2 and also strongly inhibits the activity of another receptor found in perivascular cells, PDGFR (Labrecque et al., 2005). This combined inhibitory effect of receptor tyrosine kinases that are both essential for angiogenesis leads to the inhibition of angiogenesis in both in vitro and in vivo assays (Lamy et al., 2002, 2005; Labrecque et al., 2005). As will be discussed in more details below, there is increasing evidence that the antiangiogenic effects of these phytochemicals may play a crucial role in the chemopreventive effect of these molecules.

4. Increase in Intestinal Absorption or Inhibition of Hepatic Metabolism. Another manner by which dietary-derived phytochemicals may influence tumor progression involve an indirect mechanism, in which a given molecule, with little intrinsic anticancer property, may greatly influence the biodisponibility of another phytochemical with potent anticancer activity. Perhaps the best example of this indirect synergistic mechanism is the effect of piperine, a black pepper component, on the serum concentration of curcumin. In the absence of piperine, curcumin is poorly absorbed and rapidly excreted. However, when co-administered with piperine, the absorption of curcumin is increased about a thousand-fold (Shoba et al., 1998), an effect possibly related to the modulation of intestinal proteins, such as P-gp and CYP3A4, both involved in the metabolism of xenobiotics (Zhou et al., 2004).

Nutratherapy: Using Natural Anticancer Phytochemicals to Prevent the Development of Cancer

Cancer is by no mean a spontaneous disease: in fact, tumorigenesis is a rather slow process, taking place over a long period of time during which the precancerous cells must mutate key genes involved in growth control, acquire resistance to apoptosis and trigger angiogenesis in order to grow and invade the host tissues (Hanahan and Weinberg, 2000). During this period, which may take place over several years (even several decades), tumor cells are vulnerable and are thus more susceptible to anticancer agents than mature tumors. Preventing the development of cancer by attacking these latent tumors may thus represent a very promising approach to reduce the cancer burden in our societies.

Such an approach is extremely important since even healthy individuals contain a significant number of these latent tumors in their tissues. For example, pathological studies performed on individuals deceased from causes not related to cancer have shown that 30–50% of women aged 50 had premalignant microscopic breast tumors, while 40% of similarly-aged men had precancerous cells in their prostate (Black and Welch, 1993). Even more strikingly, virtually every individuals (98%) contain small and latent tumors in the thyroid, although these tumors are only very rarely observed in the clinic. It thus

seems that the spontaneously formation of small tumors is a frequent event during lifetime but that in most cases the growth of these small tumors is tightly controlled by our natural defense mechanisms to maintain them into a microscopic and harmless state. The appearance of a clinically-detectable cancer and life-threatening cancer thus implies that the small tumors have overwhelm these natural barriers and acquired the ability to grow and invade the host tissues (Folkman and Kalluri, 2004). Using the anticancer properties of phytochemicals found in fruits and vegetables to boost our natural defenses as a mean to prevent cancer may thus represent the most effective strategy to rapidly reverse the incidence of this disease.

There are several reasons that make foods an extremely attractive source of anticancer phytochemicals to counteract the progression of cancer. First, in several cases, anticancer molecules from foods show therapeutic properties that are analogous to those of synthetic drugs but, contrarily to synthetic molecules, whose inherent toxicity limits their usage to prevention ends, the anticancer molecules present naturally in foods were selected by evolution as being beneficial for health and are therefore lacking secondary harmful effects. Secondly, this lack of toxicity implies that it is possible to use these molecules on a daily basis to prevent the development of cancer, a "metronomic" approach that is increasingly recognized as an efficient mean to restrain tumor progression (Kerbel and Kamen, 2004). Finally, dietary-derived phytochemicals interfere with several crucial aspects of tumor progression and thus increase the effectiveness of therapeutic intervention. The use of anticancer molecules present in the diet as a therapeutic weapon therefore constitutes an essential approach to maintain these tumors in a latent state and prevent their progression to cancer. This approach, that we propose to name nutratherapy, can be compared to a chemotherapy using the arsenal of anticancer molecules present in foods in order to fight the cancerous cells that spontaneously develop in our bodies. Fighting the development of cancer by the diet thus means using the anticancer molecules present in certain foods to create a hostile environment to the cancerous cells, to bombard daily these microtumors and ultimately, prevent their growth (as does chemotherapy). If the dietary habits contains a high amount of bad foods or a lack of protective foods, as fruits and vegetables, the latent tumors will be in an environment more favorable to their growth and are more likely to evolved into a cancer. On the other hand, if the diet is rich in protective foods and contains only a weak proportion of cancer-triggering foods, the microtumors will not arrive to grow sufficiently and the risks to develop a cancer will be reduced.

Beating cancer does not mean waiting for the disease to appear before fighting it but rather to circumvent its development by including in the daily diet foods that are exceptionally rich sources of natural anticancer molecules.

The Nutrinome Project: Assessing the Anticancer Properties of Fruits and Vegetables

In spite of intensive efforts to sensitize Western populations to the benefits of fruit and vegetable consumption, the daily intake of these foods still remain very low in Western countries. Moreover, the overall spectrum of consumed fruits and vegetables is rather limited: in the United States, potatoes and fruit juices, both foods with high glycemic index, account for about 30% of total per capita of fruit and vegetable consumption (Krebs-Smith, 2001). By contrast, broccoli or dark green vegetables, two rich sources of anticancer phytochemicals, make only 1% of the per capita vegetable intake. It is thus not only important to increase the overall consumption of fruits and vegetables but also to identify additional foods that can be used to diversify the diet and increase the intake of anticancer phytochemicals. No food contains by itself all the anticancer molecules being able to act on all processes involved in tumor development, highlighting the importance to integrate a large variety of foods in the diet. For example, the intake of cruciferous vegetables as well as those of the *Allium* family helps the organism to eliminate carcinogenic substances, thereby reducing their capacity to induce mutations in DNA and to favor the appearance of cancerous cells. In parallel, the absorption of green tea, berries as well as of soy prevents the formation of new blood

vessels necessary to the growth of the microtumors and maintain them in a latent state (antiangiogenic effect). Some molecules present in these foods even act on several aspects of cancer progression and maximize the protection offered by the diet. Resveratrol from grapes, for example, acts on the three processes underlying tumor development (Jang et al., 1997). Genistein from soy, in addition of being a phytoestrogen protective against hormone-dependent cancers, is also a powerful inhibitor of several proteins involved in the uncontrolled growth of cancer cells (Ravindranath et al., 2004). This variety of anticancer molecules associated with the anticancer diet is important since the growth of cancerous cells involves several distinct processes that cannot be completely inhibited by a single class of anticancer agents.

The aim of the nutrinome project is to precisely establish the anticancer properties of a wide variety of fruits and vegetables that are available to consumers in order to elaborate what could be called the optimum diet to prevent cancer. This strategy is based on the screening of fruits and vegetable extracts for their ability to act on a variety of cellular and molecular events that are crucial for tumor progression (Fig. 1). At the molecular level, these include determination of the antioxidant activity of the extracts using the $ORAC_{FL}$ assay and monitoring of the effect of the extracts on xenobiotic-metabolizing systems, with particular emphasis on cytochrome P-450 and induction of the Phase II detoxification enzymes. At the cellular level, the aim of the project is to determine the cytotoxic and antiangiogenic properties of the extracts in order to identify foods with the highest anticancer potential against several tumor cell lines. As shown in Fig. 2, there is considerable variation in the cytotoxic properties of a given food extract depending on the origin of the cancer cell line. Radish extracts, for example, potently inhibit the growth of gastric and breast tumor cells but have much less inhibitory effect on those of pancreatic, prostate or lung origin (Fig. 2). We thus believe that the precise identification of the anticancer properties of a wide variety of edible fruits and vegetables will provide crucial informations for the development of efficient dietary-based chemo-preventive approaches.

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Figures

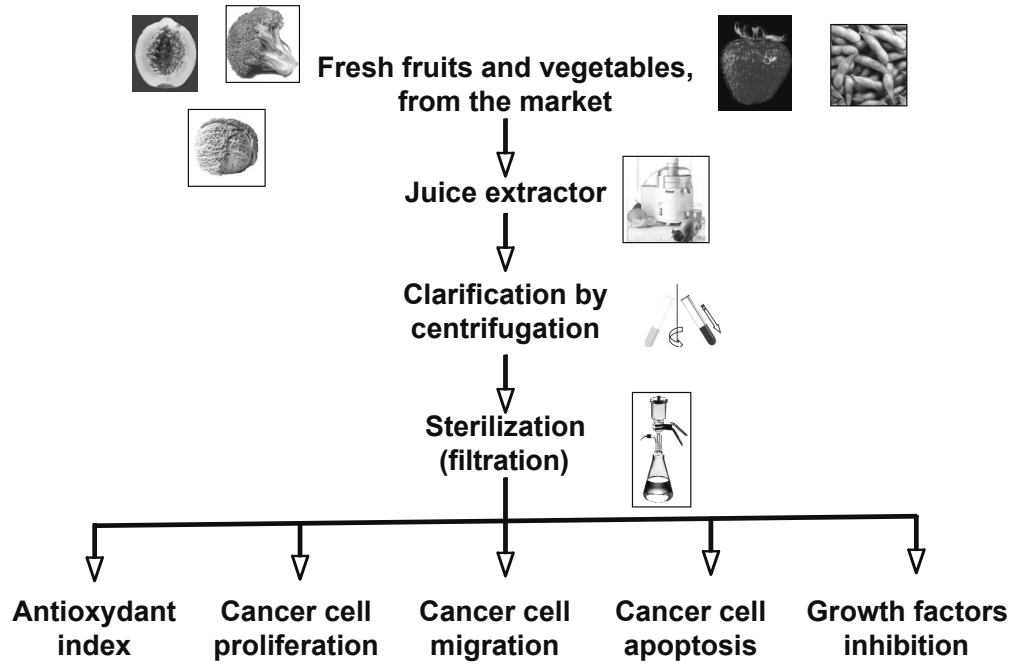

Fig. 1. Experimental approach to monitor the anticancer properties of fruits and vegetables.

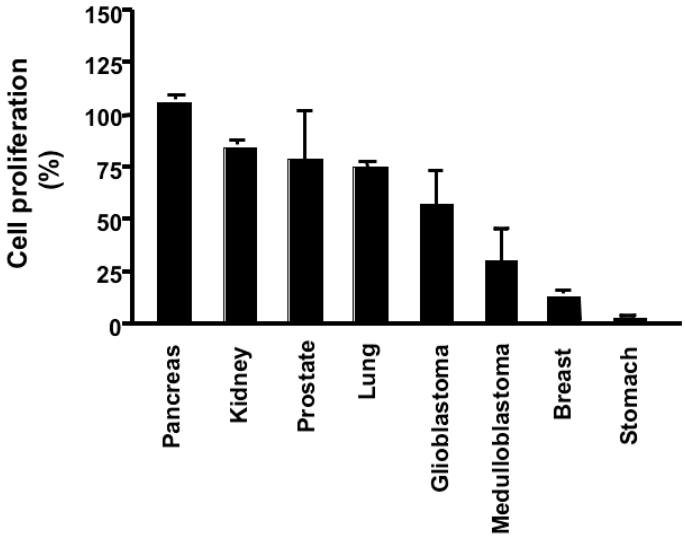

Fig. 2. Effect of radish extracts on the proliferation of tumor cell lines.