CANCER CHEMOPREVENTION WITH DIETARY PHYTOCHEMICALS

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Chemoprevention refers to the use of agents to inhibit, reverse or retard tumorigenesis. Numerous phytochemicals derived from edible plants have been reported to interfere with a specific stage of the carcinogenic process. Many mechanisms have been shown to account for the anticarcinogenic actions of dietary constituents, but attention has recently been focused on intracellular-signalling cascades as common molecular targets for various chemopreventive phytochemicals.

Cancer is a growing health problem around the world — particularly with the steady rise in life expectancy, increasing urbanization and the subsequent changes in environmental conditions, including lifestyle. According to a recent report by the World Health Organization (WHO), there are now more than 10 million cases of cancer per year worldwide. In 2003, it is estimated that approximately 1,300,000 new cases of cancer will be diagnosed, and more than 550,000 people will die from cancer in the United States alone.

Although there is no 'magic bullet' that can completely conquer cancer, many types of the disease might be avoidable. Cancer risk can be reduced by eliminating the identified carcinogens — or at least minimizing exposure to them — but, without complete identification of the corresponding risk factors, such primary prevention might be difficult to implement. Furthermore, the avoidance of some risk factors could require large lifestyle changes, which are not easy to implement.

It has been estimated that more than two-thirds of human cancers could be prevented through appropriate lifestyle modification. Richard Doll and Richard Peto have reported that 10–70% (average 35%) of human cancer mortality is attributable to diet[1]. Their observations, which are based on statistical and epidemiological data, mainly concerned dietary factors that increase risk. Although the exact percentage is uncertain, there are several lines of compelling evidence from epidemiological, clinical and laboratory studies that link cancer risk to the nutritional factors.

A wide array of substances derived from the diet have been found to stimulate the development, growth and spread of tumours in experimental animals, and to transform normal cells into malignant ones. These are regarded as suspected human carcinogens.

So, many dietary constituents can increase the risk of developing cancer, but there is also accumulating evidence from population as well as laboratory studies to support an inverse relationship between regular consumption of fruit and vegetables and the risk of specific cancers. Several organizations — such as the WHO, the American Cancer Society, the American Institute of Cancer Research (AICR) and the National Cancer Institute (NCI) — have established dietary guidelines to help people reduce the cancer risk (for further information, see the 1997 World Cancer Research Fund and AICR report in online links box).

Many clinical trials on the use of nutritional supplements and modified diets to prevent cancer are ongoing. It is conceivable that in the future people might only need to take specially formulated pills that contain substances derived from edible plants to prevent cancer or delay its onset[2]. However, a precise assessment of the mechanisms by which the components of fruit and vegetables prevent cancer is necessary before they can be recommended for inclusion in dietary supplements or before they can be tested in human intervention trials.

Phytochemicals are non-nutritive components in the plant-based diet ('phyto' is from the Greek word meaning plant) that possess substantial anticarcinogenic and antimutagenic properties. Given the great structural

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doi:10.1038/nrc1189

Summary

- Many population-based studies have highlighted the ability of macronutrients and micronutrients in vegetables and fruit to reduce the risk of cancer. Recently, attention has been focused on phytochemicals — non-nutritive components in the plant-based diet that possess cancer-preventive properties.
- Despite remarkable progress in our understanding of the carcinogenic process, the mechanisms of action of most chemopreventive phytochemicals have not been fully elucidated.
- Chemopreventive phytochemicals can block initiation or reverse the promotion stage of multistep carcinogenesis. They can also halt or retard the progression of precancerous cells into malignant ones.
- Many molecular alterations associated with carcinogenesis occur in cell-signalling pathways that regulate cell proliferation and differentiation. One of the central components of the intracellular-signalling network that maintains homeostasis is the family of mitogen-activated protein kinases (MAPKs).
- Numerous intracellular signal-transduction pathways converge with the activation of the transcription factors NF-κB and AP1. As these factors mediate pleiotropic effects of both external and internal stimuli in the cellular-signalling cascades, they are prime targets of diverse classes of chemopreventive phytochemicals.
- Basic helix–loop–helix transcription factors such as NRF2 regulate expression of phase II enzymes, which detoxify carcinogens and protect against oxidative stress. A number of phytochemicals have been shown to induce expression of phase II enzymes via NRF2.
- β-Catenin, a multifunctional protein that was originally identified as a component of cell–cell adhesion machinery, is another important molecular target for chemoprevention. Several dietary phytochemicals have been shown to target this molecule.

diversity of phytochemicals, it is not feasible to define structure–activity relationships to deduce their underlying molecular mechanisms. A better approach is to analyse their effects on cancer-associated signal-transduction pathways.

Importance of plant-derived foods

More than 250 population-based studies, including case–control and cohort studies, indicate that people who eat about five servings of fruit and vegetables a day have approximately half the risk of developing cancer — particularly cancers of the digestive and respiratory tracts — of those who eat fewer than two servings. In the United States, these observations led to the development of public-health campaigns such as the 'Five-a-Day for Better Health' programme and a more recent 'Savor the Spectrum' campaign — both were designed to increase the ingestion of fruit and vegetables by the population (BOX 1). Increased consumption of fruit and vegetables is a global priority in the prevention of cancer and other chronic disorders. According to the WHO Report 2002, there are at least 2.7 million deaths globally per year, which are primarily attributable to low fruit and vegetable intake.

Vegetables and fruit are excellent sources of cancer-preventive substances. The NCI has identified about 35 plant-based foods that possess cancer-preventive

Box 1 | **Chemoprevention initiatives**

A number of government programmes have been created in the United States and in Europe to increase vegetable consumption and decrease cancer incidence. These include the following:

The 'Five-A-Day for Better Health' programme
Founded in late 1991, this is the first nationwide health-promotion campaign to encourage people in the United States to eat fruit and vegetables — at least five servings a day — to reduce the risk of cancer and other chronic diseases. Over the past decade, there has been a steady increase in both awareness of the health benefits of fruit and vegetables and their consumption in the United States. The National Cancer Institute (NCI) has recently completed a review of this programme and reported a series of recommendations for the next round of the initiative (for further information, see the 'Five-A-Day for Better Health' report in online links box).

'Savor the Spectrum'
The NCI's spring 2002 media promotion, entitled 'Savor the Spectrum', urges all Americans to eat five to nine servings of colourful fruit and vegetables a day for better health. The message of this programme is based on current research showing that phytonutrients from different colour groups are powerful disease fighters that help our body fight off cancer and heart disease. NCI has produced a series of guidelines featuring each colour of the 'rainbow' of fruit and vegetables.

European Prospective Investigation of Cancer and Nutrition (EPIC)
EPIC is one of the most important multicentre prospective cohort studies ever launched worldwide. Beginning in 1992, EPIC has involved more than half a million (520,000) participants recruited by 20 centres in 10 countries under the coordination of the International Agency for Research on Cancer (IARC) and partly funded by the 'Europe Against Cancer' programme of the European Commission, as well as by the participating countries. EPIC focuses on identifying the dietary determinants of cancer, and is aimed at expanding the presently limited knowledge of the role of nutrition and other lifestyle factors in the aetiology and prevention of cancer and other life-threatening diseases.

Global Strategy on Dietary Prevention of Cancer
For a global extension of the 'Five-A-Day' concept of boosting increased consumption of fruit and vegetables, the WHO organized the third Biennial 'Five-A-Day' International Symposium on January 14–15 2003 in Berlin, Germany. At the meeting, Derek Yach, the WHO Executive Director of Noncommunicable Diseases & Mental Health, said "Increasing the consumption of fruit and vegetables is a necessary part of the effort to reduce the growing global burden of chronic diseases including cancer." The guidelines stated "choose most of the foods you eat from plant sources".

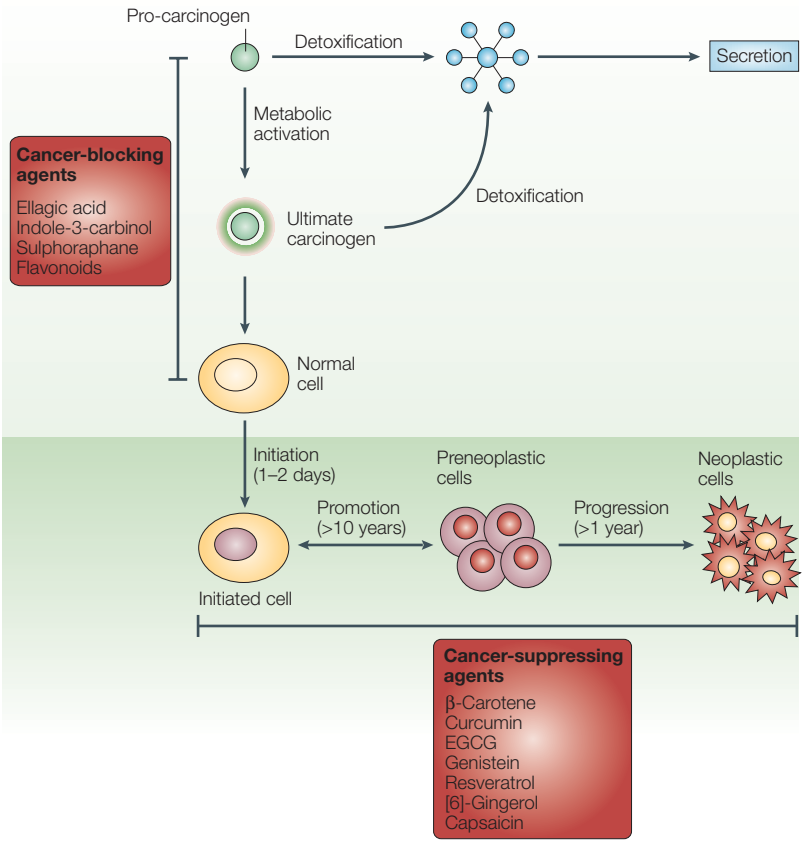

Figure 1 | **Dietary phytochemicals that block or suppress multistage carcinogenesis.** Carcinogenesis is initiated with the transformation of the normal cell into a cancer cell (initiated cell). These cells undergo tumour promotion into preneoplastic cells, which progress to neoplastic cells. Phytochemicals can interfere with different steps of this process. Some chemopreventive phytochemicals inhibit metabolic activation of the procarcinogens to their ultimate electrophilic species, or their subsequent interaction with DNA. These agents therefore block tumour initiation (blocking agents). Alternatively, dietary blocking agents can stimulate the detoxification of carcinogens, leading to their secretion from the body. Other phytochemicals suppress the later steps (promotion and progression) of multistage carcinogenesis (suppressing agents). Some phytochemicals can act as both blocking and suppressing agents. Adapted from REF. 128.

properties. These include garlic, soybeans, ginger, onion, turmeric, tomatoes and cruciferous vegetables (for example, broccoli, cabbage, cauliflower and Brussels sprouts). Numerous cell-culture and animal-model studies have been conducted to evaluate the ability of specific edible plants to prevent cancer.

Beyond vitamins to phytochemicals

Many population-based studies have highlighted the ability of macronutrients (for example, carbohydrate, proteins, fat and fibre) and micronutrients (for example, antioxidant vitamins and trace minerals) that are contained in vegetables and fruit to reduce the risk of cancer. The most exciting findings have been achieved with antioxidant vitamins and their precursors, which are found in dark, leafy green vegetables and yellow/orange fruit and vegetables. The NCI has therefore sponsored a series of human intervention trials with individual vitamins and minerals. However, plants contain numerous chemical substances other than these micronutrients that might also be useful in preventing cancer. Recently, the focus and emphasis have shifted to the non-nutritive phytochemicals. The NCI has determined in laboratory studies that more than 1,000 different phytochemicals possess cancer-preventive activity. It is estimated that there could be more than 100 different phytochemicals in just a single serving of vegetables.

As early as 1980, the NCI's Chemoprevention Programme of the Division of Cancer Prevention and Control began evaluating phytochemicals for safety, efficacy and applicability for cancer prevention. Michael Sporn coined the term 'chemoprevention' in the mid-1970s to describe the strategy of blocking or slowing the onset of premalignant tumours with relatively nontoxic chemical substances. To better define and guide research in the field of chemoprevention, the NCI Division of Cancer Prevention started the Chemoprevention Implementation Group in 1998, and then the Rapid Access to Preventive Intervention Development programme. The NCI has more than 400 potential agents under investigation and is sponsoring more than 65 Phase I, Phase II and Phase III chemoprevention trials. These involve various substances or their mixtures, many of which are foodborne phytochemicals.

Mechanisms of chemoprevention

Carcinogenesis is generally recognized as a multistep process in which distinct molecular and cellular alterations occur. From the study of experimentally induced carcinogenesis in rodents, tumour development is considered to consist of several separate, but closely linked, stages — tumour initiation, promotion and progression. Although these divisions are an oversimplification of carcinogenesis, it is useful to think in these stages when considering possible opportunities for chemoprevention.

Initiation is a rapid and irreversible process that involves a chain of extracellular and intracellular events. These include the initial uptake of or exposure to a carcinogenic agent, its distribution and transport to organs and tissues where metabolic activation and detoxification can occur, and the covalent interaction of reactive species with target-cell DNA, leading to genotoxic damage. In contrast to initiation, tumour promotion is considered to be a relatively lengthy and reversible process in which actively proliferating preneoplastic cells accumulate. Progression, the final stage of neoplastic transformation, involves the growth of a tumour with invasive and metastatic potential.

According to the conventional classification originally proposed by Lee Wattenberg, chemopreventive agents are subdivided into two main categories — blocking agents and suppressing agents[3]. Blocking agents prevent carcinogens from reaching the target sites, from undergoing metabolic activation or from subsequently interacting with crucial cellular macromolecules (for example, DNA, RNA and proteins). Suppressing agents, on the other hand, inhibit the malignant transformation of initiated cells, in either the promotion or the progression stage. Chemopreventive phytochemicals can block or reverse the premalignant stage (initiation and promotion) of multistep carcinogenesis. They can also halt or at least

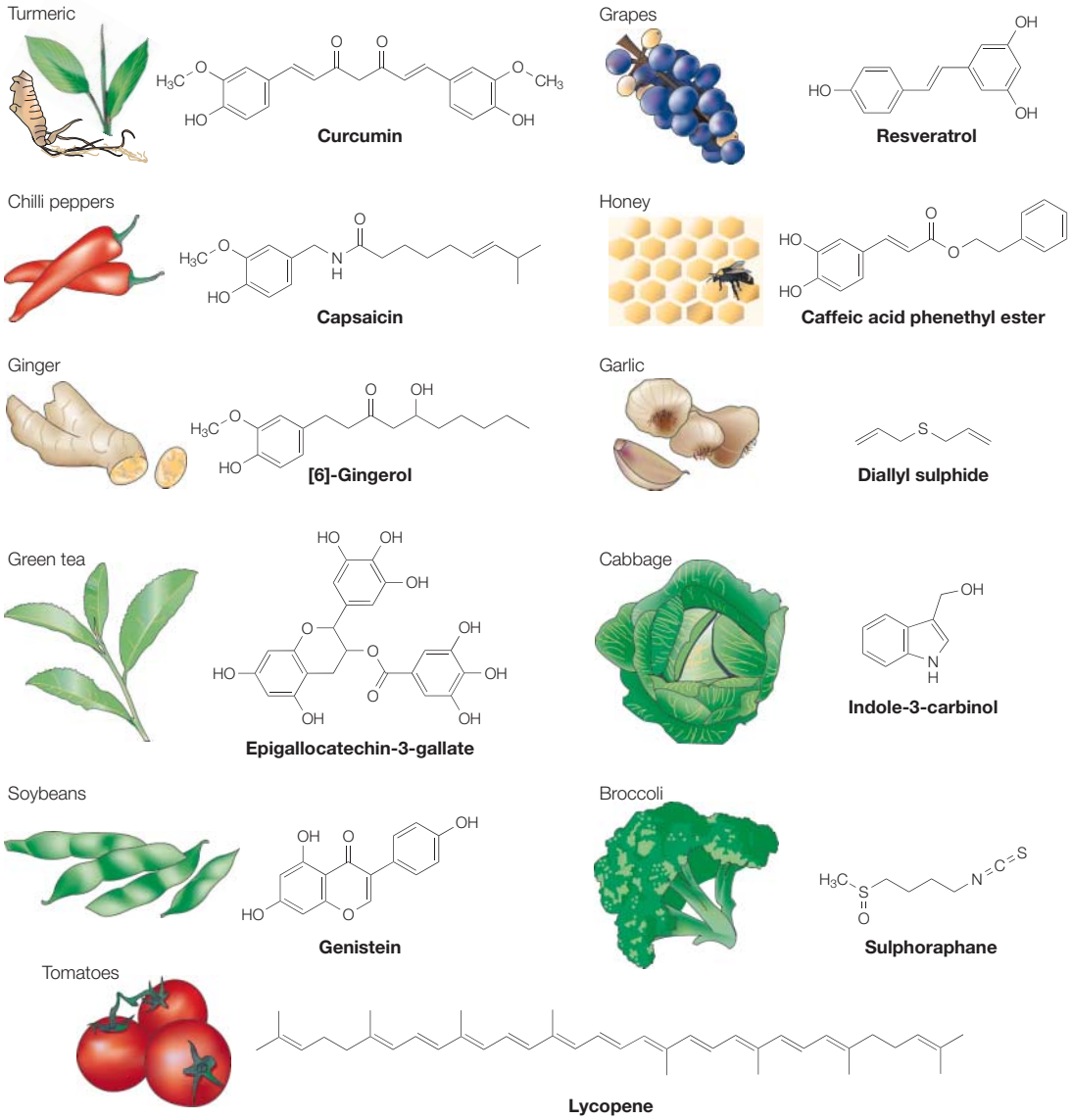

Figure 2 | **Representative chemopreventive phytochemicals and their dietary sources.**

retard the development and progression of precancerous cells into malignant ones (FIG. 1). Recent advances in our understanding of the carcinogenic process at the cellular and molecular level have shown this blocking and suppressing categorization to be an oversimplification, and numerous cellular molecules and events that could be potential targets of chemopreventive agents have been more specifically identified[4–6]. Therefore, the ability of any single chemopreventive phytochemical to prevent tumour development should be recognized as the outcome of the combination of several distinct sets of intracellular effects, rather than a single biological response.

FIGURE 2 illustrates the chemical structures of representative dietary phytochemicals that have been known to possess chemopreventive potential and their dietary sources. The cellular and molecular events affected or regulated by these chemopreventive phytochemicals include carcinogen activation/detoxification by xenobiotic metabolizing enzymes; DNA repair; cell-cycle progression; cell proliferation, differentiation and apoptosis; expression and functional activation of oncogenes or tumour-suppressor genes; angiogenesis and metastasis; and hormonal and growth-factor activity (for further information, see ONLINE TABLE 1).

Cellular signalling molecules as targets

During the past two or three decades, there has been substantial progress in identifying the biochemical events that are associated with the multistage process of carcinogenesis, and we are now better aware of how certain dietary phytochemicals are able to alter this process (FIG. 1). Remarkable advances in the cellular and molecular genetics of carcinogenesis — such as the identification of numerous oncogenes and tumour-suppressor genes, specific genes encoding

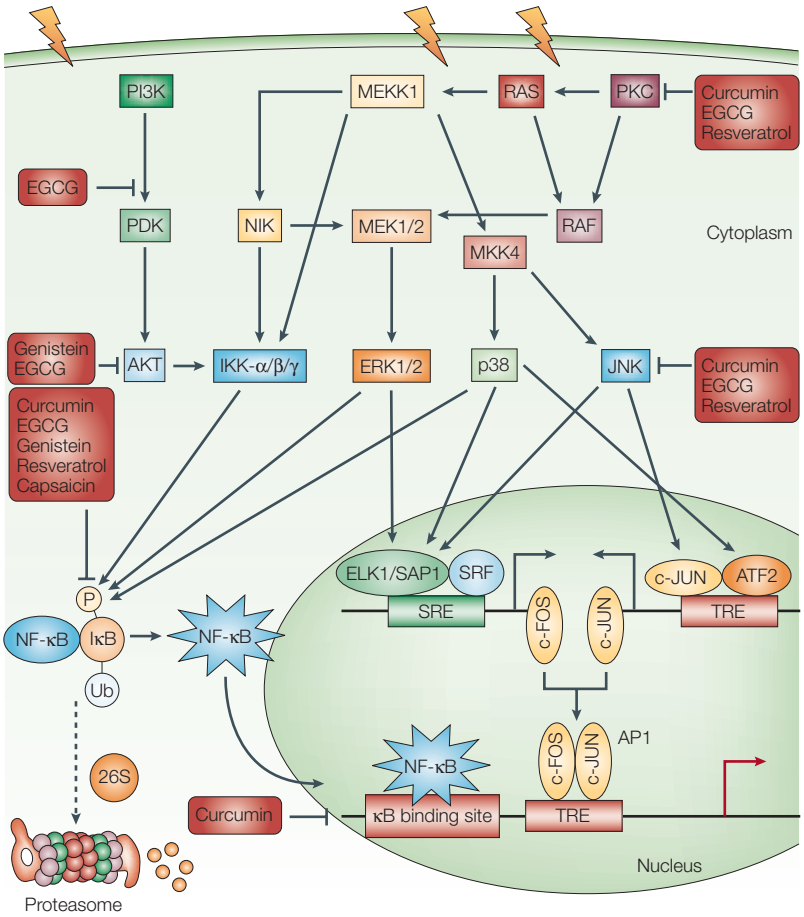

Figure 3 | **Effect of phytochemicals on activation of NF-κB and AP1.** The NF-κB signalling pathway converges on the multiprotein complex called the IκB kinase (IKK) signalsome, leading to IκB phosphorylation (P), ubiquitylation (Ub) and subsequent degradation by the 26S proteasome. NF-κB is then released and translocated to the nucleus, where it binds to specific promoter regions of various genes. The IKK signalsome is activated by the NF-κB-inducing kinase (NIK). Pathways that regulate NIK are likely to involve signalling through a family of mitogen-activated protein kinases (MAPKs), such as MAPK kinase kinase-1 (MEKK1) — a kinase that lies upstream of extracellular signal-regulated kinase (ERK) — MAPK/ERK kinase (MEK1/2) and p38 MAPK. Recent reports showed that NF-κB activation is also regulated by the AKT signalling pathway[58,59,129]. Phosphatidylinositol 3-kinase (PI3K) activates AKT/protein kinase B via phosphorylation by 3-phosphoinositide-dependent protein kinase-1 (PDK1). Genistein specifically inhibits AKT activity and AKT-mediated NF-κB activation[58,59]. Epigallocatechin gallate (EGCG) can block the activities of PI3K and AKT[49]. There is crosstalk between the AKT and NF-κB signalling pathways — AKT phosphorylation leads to activation of NF-κB by stimulating IκB kinase (IKK) activity[129]. IKK is also a target for chemopreventive phytochemicals, including curcumin[24,28], resveratrol[71] and EGCG[45,130]. The MAPK family proteins also regulate expression of AP1 — a heterogenous set of dimeric proteins made up of members of the c-JUN, c-FOS and ATF families. In this pathway, activation of ERK1/2 phosphorylates ELK1, c-JUN NH_2-terminal kinase (JNK) phosphorylates c-JUN, and p38 phosphorylates both ELK1 and ATF2. This leads to transcriptional activation of target genes. External stimuli — including phorbol ester and ultraviolet radiation — activate specific isoforms of protein kinase C (PKC), which, in turn, leads to stimulation of the p21 RAS–ERK signalling pathway via RAF and MEK1/2. Activation of p38 and JNK is mediated by MAPK kinase-4 (MKK4), which is under control of the upstream kinase MEKK.

carcinogen-metabolizing enzymes, DNA-repair enzymes and proteins, and regulators of cell cycle and apoptosis — have given us a better insight into the process of neoplastic transformation. Advances have also been made in identifying the factors that mediate tumour invasion, metastasis and angiogenesis.

Despite this progress, the identification of molecular and cellular targets of chemopreventive phytochemicals is still incomplete. Many of the molecular alterations that are associated with carcinogenesis occur in cell-signalling pathways that regulate cell proliferation and differentiation. One of the central components of the intracellular-signalling network that maintains homeostasis is the family of proline-directed serine/threonine kinases — the mitogen-activated protein kinases (MAPKs; FIG. 3).

Abnormal or improper activation or silencing of the MAPK pathway or its downstream transcription factors can result in uncontrolled cell growth, leading to malignant transformation. Some phytochemicals 'switch on' or 'turn off' the specific signalling molecule(s), depending on the nature of the signalling cascade they target, preventing abnormal cell proliferation and growth[4–12]. Cell-signalling kinases other than MAPKs, such as protein kinase C (PKC) and phosphatidylinositol 3-kinase (PI3K), are also important targets of certain chemopreventive phytochemicals. These upstream kinases activate a distinct set of transcription factors, including nuclear factor κB (NF-κB) and activator protein 1 (AP1; FIG. 3).

NF-κB and AP1

Numerous intracellular signal-transduction pathways converge with the activation of the transcription factors NF-κB and AP1, which act independently or coordinately to regulate target-gene expression (FIG. 3).

Aberrant activation of NF-κB has been associated with protection against apoptosis and stimulation of proliferation in malignant cells[13,14], and overexpression of NF-κB is causally linked to the phenotypic changes that are characteristic of neoplastic transformation[15]. Many chemopreventive phytochemicals that are derived from the diet have been shown to suppress constitutive NF-κB activation in malignant cells or NF-κB activation induced by the external tumour promoter phorbol 12-myristate 13-acetate (PMA) or tumour-necrosis factor-α (TNF-α)[11,16,17].

AP1 is another transcription factor that regulates expression of genes that are involved in cellular adaptation, differentiation and proliferation. Functional activation of AP1 is associated with malignant transformation as well as tumour promotion[18–21]. AP1 consists of either homo- or heterodimers between members of the JUN and FOS families, which interact via a leucine-zipper domain. This transcription factor is also regulated by the MAPK-signalling cascade[21–23].

As NF-κB and AP1 are ubiquitous eukaryotic transcription factors that mediate pleiotropic effects of both external and internal stimuli in the cellular-signalling cascades, they are prime targets of diverse classes of chemopreventive phytochemicals (FIG. 3).

Phytochemicals targeting NF-κB and AP1

Curcumin, [6]-gingerol and capsaicin. Curcumin — a yellow pigment that is present in the rhizome of turmeric (*Curcuma longa* L.) and related species — is one of the most extensively investigated phytochemicals, with regard to chemopreventive potential. Curcumin

has been shown to suppress tumour promotion in a mouse model of skin carcinogenesis. Furthermore, pre-treatment of human colonic epithelial cells with curcumin inhibited TNF-α-induced cyclooxygenase-2 (*COX2*) gene transcription and NF-κB activation[24]. In this study, curcumin inhibited IκB degradation by downregulation of NF-κB-inducing kinase (NIK) and IκB kinase (IKK)α/β.

When curcumin was applied topically to the dorsal skin of female ICR mice (a model initially developed at the Institute of Cancer Research, Fox Chase Cancer Center), it prevented the PMA-induced activation of both Nf-κb and Ap1 (REF. 25). The inhibition of Nf-κb was accompanied by blockade of degradation via phosphorylation of Iκbα and also by reduced nuclear translocation of the p65 subunit of Nf-κb (REF. 26; FIG. 3). Topically applied curcumin inhibited the catalytic activity of epidermal extracellular-signal-regulated kinase (Erk)1/2, which could account for its ability to inactivate Nf-κb and Cox2 (REF. 26). Curcumin also suppressed the TNF-α-induced nuclear translocation and DNA binding of NF-κB in a human myeloid leukaemia cell line by blocking phosphorylation and subsequent degradation of IκB[27]. PMA- and hydrogen-peroxide-induced activation of NF-κB was similarly attenuated by curcumin treatment. In addition, curcumin inhibited IκBα phosphorylation in human multiple myeolma cells[28] and murine melanoma cells[29] through suppression of IKK activity, which contributed to its antiproliferative, proapoptotic and/or antimetastatic activities.

[6]-Gingerol — a phenolic substance that is responsible for the spicy taste of ginger (*Zingiber officinale* Roscoe) — was reported to inhibit tumour promotion and PMA-induced ornithine decarboxylase (ODC) activity and Tnf-α production in mouse skin[30]. More recently, [6]-gingerol has been found to inhibit epidermal growth factor (Egf)-induced Ap1 activation and neoplastic transformation in mouse epidermal JB6 cells — this was shown using reduced anchorage-independent formation of cell colonies in soft agar[31].

Capsaicin — a pungent component of hot chilli pepper (*Capsicum annuum* L.) — has been suspected to act as a carcinogen or a co-carcinogen in experimental animals because of its irritant properties, but other studies indicate that the compound has chemopreventive and chemoprotective effects[32–35]. Topical application of capsaicin inhibited PMA-induced mouse-skin tumour formation[36] and activation of Nf-κb[37]. This was attributed to blockade of Iκbα degradation and Nf-κb translocation into the nucleus. PMA- or Tnf-α-induced Ap1 activation in mouse skin and cultured human leukaemia HL-60 cells was also blocked by capsaicin[38].

Capsaicin inhibited constitutive and induced activation of NF-κB in human malignant-melanoma cells, leading to inhibition of melanoma-cell proliferation[39]. Capsaicin also induced apoptosis in cultured Jurkat cells through generation of reactive oxygen species (ROS) and rapid activation of c-JUN NH_2-terminal kinase (JNK)[40]. Similarly, capsaicin caused apoptotic death in *HRAS*-transformed human mammary epithelial cells, which was accompanied by marked activation of JNK and p38, and deactivation of ERK[41]. Pharmacological inhibition or dominant-negative forms of JNK and p38, but not of ERK, abrogated the capsaicin-induced apoptosis in these cells[41].

Epigallocatechin gallate (EGCG). EGCG is an antioxidant and chemopreventive polyphenol that is found in green tea. It has been shown to suppress malignant transformation in a PMA-stimulated mouse epidermal JB6 cell line, which seemed to be mediated by blocking activation of Ap1 (REFS 42,43) or Nf-κb[44]. More recently, EGCG treatment of human epidermal keratinocytes resulted in significant inhibition of ultraviolet (UV)-B-light-induced activation of IKKα, phosphorylation and subsequent degradation of IκBα and nuclear translocation of p65 (REF. 45). In the Hras-transformed epidermal JB6 cells, EGCG inhibited Ras-activated Ap1 activity[46,47]. Similar Ap1 inhibition was observed in the epidermis of transgenic mice that harbour an Ap1-driven luciferase reporter gene.

Nomura and colleagues[48] have reported the inhibitory effect of EGCG on UV-light-induced PI3K activation in mouse epidermal cells. The reduction of signalling via PI3K–AKT–NF-κB by EGCG was reported to be mediated through inhibition of ERBB2 (also known as HER2/NEU) receptor tyrosine phosphorylation[49]. EGCG also inhibited vascular endothelial growth factor (VEGF) production by inhibiting constitutive activation of both STAT3 and NF-κB — but not of ERK or AKT — in human breast and head and neck cancer cell lines[50].

EGCG treatment resulted in inhibition of cell growth, G_0/G_1-phase arrest of the cell cycle and induction of apoptosis in human epidermoid carcinoma (A431) cells, but not in normal human epidermal keratinocytes (NHEK)[51]. A431 cells were more susceptible to EGCG-mediated inhibition of constitutive NF-κB expression and activation than NHEK cells, indicating that EGCG-caused cell-cycle deregulation and apoptosis of cancer cells might be mediated through NF-κB inhibition. The roles of EGCG and other tea polyphenols on cellular signalling have been reviewed recently[52,53].

Genistein. Genistein — a soy-derived isoflavone — is believed to contribute to the putative breast- and prostate-cancer-preventive activity of soya. Genistein inhibited PMA-induced AP1 activity, expression of c-FOS and ERK activity in certain human mammary cell lines[54]. Genistein treatment abrogated NF-κB DNA binding in human hepatocarcinoma cells stimulated with hepatocyte growth factor[55]. The downregulation of c-Jun and c-Fos by genistein was also observed in UV-light-stimulated skin of SENCAR (sensitivity to carcinogenesis) mice[56].

Genistein at the apoptogenic concentration also inhibited the H_2O_2- or TNF-α-induced activation of NF-κB in both the androgen-sensitive (LNCaP) and -insensitive (PC3) human prostate cancer cell lines by reducing phosphorylation of IκBα and the nuclear translocation of NF-κB[57]. Genistein-mediated inactivation of NF-κB was associated with downregulation of AKT in

the prostate cancer[58] and mammary cancer[59] cells. The same studies also revealed that AKT transfection led to the activation of NF-κB, which was completely blocked by genistein treatment, indicating that inhibition of the crosstalk between AKT and NF-κB could provide a novel mechanism responsible for pro-apoptotic activity of genistein.

PMA- or TNF-α-induced NF-κB DNA binding and NF-κB-derived COX2 promoter activity, as well as COX2 expression, were inhibited in human alveolar epithelial carcinoma cells by genistein treatment[60]. In human U937 monocytes, genistein exerted no substantial inhibitory effect on DNA binding of NF-κB, but markedly attenuated its transcriptional activity[61]. Consistent with this notion, genistein strongly suppresses NF-κB transcriptional activity in PMA-stimulated human mammary epithelial cells, as determined by the LUCIFERASE-REPORTER-GENE ASSAY but does not interfere with IκB degradation, and subsequent nuclear translocation and DNA binding of NF-κB (M.-H. Chung and Y.-J.S., unpublished observations). Genistein might block the phosphorylation of p65 without influencing the IKK activity, thereby hampering its interaction with co-activators such as cyclic AMP response element binding protein (CREB)-binding protein (CBP/p300), a key element of the transcription-initiation complex that bridges DNA-bound transcription factors to the transcription machinery.

Resveratrol. Resveratrol (3,4′,5-trihydroxy-*trans*-stilbene) is a phytoalexin that is present in grapes (*Vitis vinifera*) and a key antioxidant ingredient of red wine. It is believed to be responsible for the so-called 'French paradox', in which consumption of red wine has been shown to reduce the mortality rates from cardiovascular diseases and certain cancers. Resveratrol treatment inhibited PMA-induced *COX2* expression and catalytic activity, via the cyclic-AMP response element (CRE), in human mammary epithelial cells[62,63]. It also inhibited PKC activation, AP1 transcriptional activity and the induction of *COX2*-promoter activity in PMA-treated cells. Resveratrol induced apoptosis and reduced the constitutive activation of NF-κB in both rat and human pancreatic carcinoma cell lines[64]. Mammary tumours isolated from rats treated with resveratrol displayed reduced expression of *Cox2* and matrix metalloproteinase (*Mmp*)-9, as well as reduced *Nf-κb* activation, compared with controls[65]. Treatment of human breast cancer MCF-7 cells with resveratrol also suppressed NF-κB activation and proliferation[65].

Treatment of androgen-sensitive prostate cancer cells (LNCaP) with resveratrol caused downregulation of prostate-specific antigen and p65; these effects were associated with activation of p53, WAF1, p300/CBP and APAF1 (REF. 66). Resveratrol-induced apoptosis in mouse JB6 epidermal cells was associated with phosphorylation of p53, which seemed to be mediated through activation of Erk and p38 (REF. 67). Yu and colleagues have shown that resveratrol pretreatment gives rise to suppression of PMA- and UV-light-induced activation of AP1 and MAPKs (ERK2, JNK and p38) in HeLa cell cultures, which was associated with inhibition of PKC and protein tyrosine kinase[68]. Similarly, resveratrol blocked UV-light-induced activation of NF-κB through suppression of IKK activation[69]. Resveratrol suppressed TNF-α-induced phosphorylation and nuclear translocation of p65, and NF-κB-dependent reporter-gene transcription in myeloid leukaemia cells[70]. The suppression of NF-κB coincided with suppression of AP1. Resveratrol also inhibited the TNF-induced activation of MAPK kinase (MEK) and JNK, and abrogated TNF-induced caspase activation[70].

Resveratrol induced apoptosis in fibroblasts after the induced expression of oncogenic HRAS, possibly through inhibition of NF-κB activation by blocking IKK activity[71].

Miscellaneous phytochemicals. In addition to the aforementioned phytochemicals, caffeic acid phenethyl ester (CAPE), sulphoraphane, silymarin, apigenin, emodin, quercetin and anethole have also been reported to suppress the activation of NF-κB and AP1, which might contribute to their chemopreventive and/or cytostatic effects[16].

NRF–KEAP1 complex

Other than suppressing tumour promotion or progression, another important approach to chemoprevention is to block the DNA damage caused by carcinogenic insult — the initiation stage of carcinogenesis. Toxic xenobiotic ('*xeno*', from the Greek word meaning 'foreign') chemicals, including carcinogens, are detoxified by PHASE II ENZYMES — such as glutathione *S*-transferase (GST) and NAD(P)H:quinone oxidoreductase (NQO).

The phase II enzyme induction system is an important component of the cellular stress response in which a diverse array of electrophilic and oxidative toxicants can be removed from the cell before they are able to damage the DNA. Antioxidants exert their protective effects not only by scavenging ROS, but also by inducing *de novo* expression of genes that encode detoxifying/defensive proteins, including phase II enzymes. Many xenobiotics activate stress-response genes in a manner similar to that achieved by antioxidants. These genes encode enzymes such as glutathione peroxidase, gamma-glutamylcysteine synthetase (γ-GCS), GST, NQO and heme oxygenase-1 (HO-1). The 5′-flanking regions of these genes contain a common *cis*-element, known as the ANTIOXIDANT-RESPONSIVE ELEMENT (ARE) (FIG. 4). Many basic leucine zipper (bZIP) transcription factors — including NRF, JUN, FOS, FRA, MAF and AH receptor — bind to these ARE sequences and modulate expression of some of the aforementioned stress-response genes[72] (FIG. 4).

NRF. During oxidative stress or other types of toxic insult that are induced by xenobiotic chemicals, certain members of the helix–loop–helix bZIP family of transcription factors — particularly the nuclear factor-erythroid 2p45 (NF-E2)-related factors (NRF1 and NRF2) — heterodimerize and bind to the ARE

LUCIFERASE-REPORTER-GENE ASSAY
A recombinant method that is used to measure transcriptional activity in which the regulatory sequence (for example, promoter or enhancer) of interest is joined to a firefly luciferase gene that, following activation, produces light from luciferin in the presence of ATP added to the assay mixture. The relative intensity of the light emission is measured with a luminometer.

CREB
(Cyclic AMP response element binding protein). CREB is a leucine zipper transcription factor that binds to DNA at the cyclic AMP response element (CRE) as a homo- or heterodimer. It has pivotal roles in the control of cellular proliferation and differentiation, apoptosis, intermediary metabolism, inflammation and numerous other responses, particularly in hepatocytes, adipocytes and haematopoietic cells.

PHASE II ENZYMES
A group of xenobiotic metabolizing enzymes that are mainly involved in the inactivation and excretion of carcinogens and other toxic chemical substances.

ANTIOXIDANT-RESPONSIVE ELEMENT
(ARE). A specific DNA-promoter-binding region that can be transcriptionally activated by numerous antioxidants and/or electrophiles. Many stress-response genes encoding phase II detoxification or antioxidant enzymes such as glutathione *S*-transferase, quinone reductase, and heme oxygenase-1 — which provide defence against cellular oxidative stress — have this element in their 5′-flanking region to facilitate the transcription process.

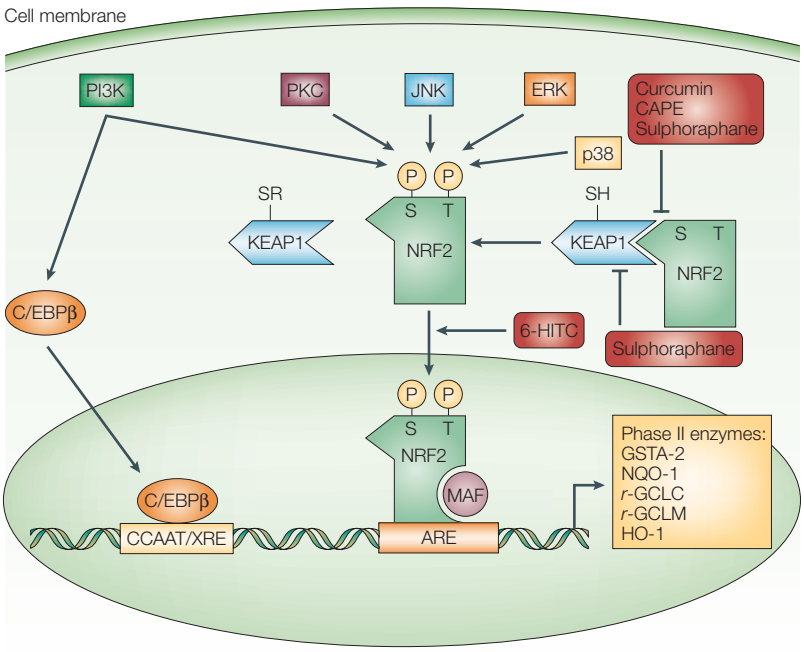

Figure 4 | **Transcriptional activation by NRF2.** NRF2 is a transcription factor that regulates expression of many detoxification or antioxidant enzymes. The Kelch-like-ECH-associated protein 1 (KEAP1) is a cytoplasmic repressor of NRF2 that inhibits its ability to translocate to the nucleus. These two proteins interact with each other through the double glycine-rich domains of KEAP1 and a hydrophilic region in the NEH2 domain of NRF2. KEAP1 contains many cysteine residues. Phase II enzyme inducers and/or prooxidants can cause oxidation or covalent modification (R) of these cysteine residues[91]. As a result, NRF2 is released from KEAP1. In addition, phosphorylation of NRF2 at serine (S) and threonine (T) residues by kinases such as phosphatidylinositol 3-kinase (PI3K), protein kinase C (PKC)[131], c-Jun NH_2-terminal kinase (JNK) and extracellular-signal-regulated kinase (ERK) is assumed to facilitate the dissociation of NRF2 from KEAP1 and subsequent translocation to the nucleus. p38 can both stimulate and inhibit the NRF2 nuclear translocation. In the nucleus, NRF2 associates with small MAF (the term is derived from musculoaponeurotic-fibrosarcoma virus), forming a heterodimer that binds to the antioxidant-responsive element (ARE) to stimulate gene expression. NRF2/MAF target genes encode phase II detoxification or antioxidant enzymes such as glutathione *S*-transferase α2 (GSTA2), NAD(P)H:quinone oxidoreductase (NQO1), γ-glutamate cysteine ligase (γ -GCLC and γ -GCLM) and heme oxygenase-1 (HO-1). PI3K also phosphorylates the CCAAT/enhancer binding protein-β (C/EBPβ), inducing its translocation to the nucleus and binding to the CCAAT sequence of C/EBP-β response element within the xenobiotic response element (XRE), in conjunction with NRF2 binding to ARE[132]. Transfection of human neuroblastoma cells with PI3K activates ARE, which is attenuated by a pharmacological inhibitor of PI3K or dominant-negative NRF2 (REF. 133). Curcumin and caffeic acid phenethyl ester (CAPE) disrupt the NRF2–KEAP1 complex, leading to increased NRF2 binding to ARE[99,100]. Sulphoraphane directly interacts with KEAP1 by covalent binding to its thiol groups[91]. 6-(Methylsulfinyl)hexyl isothiocyanate (6-HITC) — a sulphoraphane analogue from Japanese horseradish wasabi — stimulates nuclear translocation of NRF2, which subsequently activates ARE[98].

sequence to activate transcription[73]. In human hepatoma cells that are genetically engineered to overexpress *NRF1* or *NRF2*, both basal and inducible transcriptional activities of an *ARE* reporter gene were increased.

A role for NRF2 in the regulation of ARE-mediated gene expression has been shown in studies involving *Nrf2*-null mice[73]. These mice fail to induce many of the genes involved in carcinogen detoxification and protection against oxidative stress[73–83]. Most notably, the *Nrf2*-null mice developed a larger number of tumours in the forestomach after treatment with the ubiquitous carcinogen benzo[*a*]pyrene, which was not prevented by oltipraz, a chemopreventive agent with phase II enzyme inducing activity[75,84]. *Nrf2*-null mice also have defects in detoxifying carcinogens such as aflatoxin B_1[85]. Stable transfection of L929 cells with a dominant-negative mutant form of Nrf2 abolished induction of Ho-1 by several toxicants[86]. Fibroblasts from *Nrf2*-null mice were found to express only about 15% as much *Gcs* mRNA as wild-type cells[87]. Overexpression of NRF2 activated ARE-mediated transcription in human hepatoma (HepG2) cells, and this activation was further increased by *tert*-butylhydroquinone[88].

KEAP1 — a negative regulator of NRF. A cytosolic actin-binding protein called Kelch-like ECH-associated protein 1 (KEAP1) has been identified as a docking site at which the bZIP proteins are sequestered under normal physiological conditions. For example, KEAP1 suppresses the transcriptional activity of NRF2 by retaining the transcription factor in the cytoplasm and hampering its nuclear translocation (FIG. 4).

The mechanisms by which cells recognize chemopreventive antioxidants or phase II enzyme inducers have not been fully elucidated. The KEAP1–NRF2 complex is an intracellular sensor that recognizes redox signalling by detecting electrophiles or ROS[89]. Many phase II gene inducers are able to generate ROS, or else can be readily converted — nonenzymatically, via REDOX CYCLING — or metabolized to electrophilic intermediates in the body. Phase II enzyme inducers mimic pro-oxidants and electrophiles, although most of them are antioxidants by nature. Therefore, it might be more appropriate to call ARE an 'electrophile response element' (EpRE). It is plausible that these reactive species interact with thiol groups of KEAP1 and oxidize or covalently modify the cysteine residues within KEAP1 and also, possibly, NRF2 (REFS 90–93). This would cause KEAP1 to release NRF2, so it could translocate to the nucleus and activate transcription of phase II enzymes (FIG. 4).

In accordance with this model, sulphydryl-reactive agents — such as diethyl maleate —abrogated KEAP1 repression of NRF2, allowing release of the transcription factor[89]. In this context, the cysteine residues in KEAP1 could serve as a molecular sensor of intracellular redox status, ensuring the proper and timely expression of genes that are involved in cellular antioxidant defence or detoxification of electrophilic toxicants.

Phytochemicals that activate NRF

Exposure of HepG2 cells to the green-tea extract induces expression of phase II detoxifying enzymes through ARE[94]. This upregulation was accompanied by activation of ERK2 and JNK1, as well as immediate-early genes c-*JUN* and c-*FOS*. Subsequent studies have shown that EGCG transcriptionally activated the phase II enzyme gene expression in HepG2 cells, as determined by the ARE reporter-gene assay[95]. In this experiment, EGCG strongly activated all three MAPKs (ERK, JNK and p38) and induced caspase-3-mediated

REDOX CYCLING
A reciprocal transformation between an oxidant and its reductive counterpart. An example is conversion of catechol to quinone via semiquinone or *vice versa*.

cell death. Other phytochemicals such as phenethyl isothiocyanate and sulphoraphane also differentially regulated the activation of MAPKs and NRF, ARE-mediated luciferase reporter-gene activity, and phase II enzyme gene induction[96,97].

Analysis of gene-expression profiles by an oligonucleotide microarray revealed that sulphoraphane upregulated expression of Nqo1, Gst and Gcs in the small intestine of wild-type mice, whereas the *Nrf2*-null mice displayed much lower levels of these enzymes[80]. During extensive screening of vegetable extracts for GST-inducing activity in cultured rat liver epithelial RL-34 cells, Morimitsu and colleagues have identified a sulphoraphane analogue, 6-methylsulphinylhexyl isothiocyanate (6-HITC), as a key GST-inducer present in Japanese horseradish, wasabi (*Wasabia japonica* or *Eutrema wasabi* Maxim)[98]. The compound potently induced both class α Gsta1 and class π Gstp1 isozymes in RL-34 cells by stimulating nuclear translocation of Nrf2 and subsequent activation of Are. Oral administration of 6-HITC resulted in the induction of hepatic phase II detoxification enzymes to a greater extent than sulphoraphane, whereas this induction was abrogated in *Nrf2*-null mice[98]. In porcine renal epithelial cells, both curcumin and CAPE stimulated expression of *Nrf2* by inactivating the Nrf2–Keap1 complex, which was associated with a significant increase in activity and expression of Ho-1 (REF. 99; FIG. 4). p38 Mapk, which is upstream of Nrf2, seems to be involved in curcumin-induced *Ho1* gene induction. In another study, curcumin increased nuclear translocation of Nrf2, Are DNA binding activity and GCL expression[100]. It is notable that both curcumin and CAPE bear an α, β-unsaturated ketone moiety, and can therefore act as Michael-reaction acceptors that are able to modify cysteine thiols located in Keap1. Sulphoraphane also directly reacts with thiol groups of Keap1 (REF. 91).

β-Catenin

β-Catenin is another important target of chemopreventive phytochemicals. β-Catenin is a multifunctional protein that was originally identified as a component of the cell–cell adhesion machinery. It binds with the cytosolic tail of E-cadherin and connects actin filaments through α-catenin to form the cytoskeleton[101,102] (FIG. 5). It was identified as a component of the evolutionarily conserved WNT signalling pathway, and is involved in developmental processes in many organisms, as well as in tumorigenesis. β-Catenin can also function as a transcription factor, and nuclear translocation of β-catenin has been associated with various human cancers[103].

The cytoplasmic β-catenin undergoes rapid turnover by a large multiprotein complex that consists of glycogen synthase kinase-3β (GSK-3β), adenomatous polyposis coli (APC), axin and conductin[104,105]. GSK-3β — either directly or through activation of APC — phosphorylates β-catenin, leading to ubiquitylation followed by proteasomal degradation of β-catenin[104–106]. Recently, the phosphorylation of the serine-45 residue of β-catenin by casein kinase Iε (CKI) has been shown to convert β-catenin into a form that is favoured for phosphorylation by GSK-3β, and so promotes destabilization of β-catenin[107,108]. Liu *et al.* reported a similar function of another isoform of casein kinase, CKIα[109].

So, β-catenin needs to be stabilized in the cytoplasm to escape the degradation pathway. This occurs in response to WNT signalling, as well as signalling by several growth factors, such as platelet-derived endothelial factor and bacterial lipopolysaccharide. GSK-3β can be inactivated by phosphorylation of serine-9, either through WNT signalling or through activation of the PI3K–AKT pathway[110]. Stabilization of β-catenin also occurs in the case of either mutation of APC[111] or axin[112]. In addition, a point mutation at the phosphorylation site of the amino-terminal domain of β-catenin turns it into an oncoprotein[103] that is resistant to phosphorylation by GSK-3β.

Once β-catenin is stabilized, it translocates into the nucleus and interacts with lymphoid enhancer factor (LEF)/T-cell factor (TCF) transcription factors, resulting in transcriptional activation of various genes. Many of these gene products are involved in processes such as cell-cycle regulation, cell adhesion and cellular development[103,113]. Genes that undergo transactivation mediated by the β-catenin–TCF/LEF complex include those encoding c-MYC, cyclin-D1, gastrin, human matrilysin (MMP7), keratin1, urokinase plasminogen-activated receptor (uPAR), CD44 and ITF2 (REFS 114,115). Transcription factors such as c-JUN and FRA1 — two components of AP1 — are reported to be regulated by the transcriptional activity of the β-catenin–TCF/LEF complex[103]. Recently, a TCF4-binding element (TBE) has been identified in the COX2-promoter region, and the β-catenin–TCF/LEF complex has been shown to upregulate *COX2* gene expression in human colorectal HT29-APC cells[116].

Phytochemicals that target β-catenin

Several dietary phytochemicals have been shown to downregulate the β-catenin-mediated signalling pathway as part of their molecular mechanism of chemoprevention. Curcumin and CAPE inhibited tumorigenesis and decreased β-catenin expression in the multiple intestinal neoplasia (Min/+) mouse model[117]. Moreover, curcumin reduced the cellular leves of β-catenin through caspase-mediated cleavage of the protein[118]. Downregulation of β-catenin expression by resveratrol was observed in a human colon cancer cell line[119]. Expression of a β-catenin–TCF4-binding reporter construct was reduced in HEK293 cells by EGCG[120,121]. Indole-3-carbinol altered the pattern of β-catenin mutation in chemically-induced rat colon tumours[122], inhibited adhesion, migration and invasion of cultured human breast carcinoma cells, and upregulated E-cadherin and β-catenin[123]. A similar effect was observed with tangeretin from citrus[124]. COX inhibitors have also been found to suppress β-catenin signalling and β-catenin–TCF/LEF transcriptional activity[125–127].

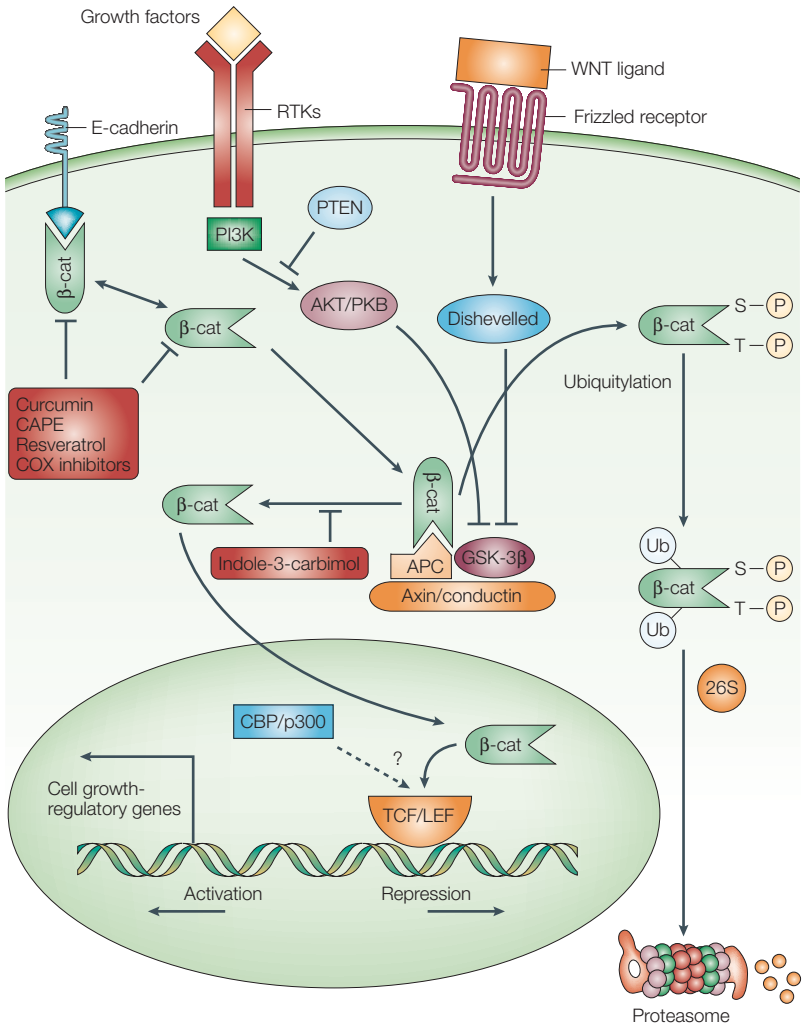

Figure 5 | **Effect of phytochemicals on β-catenin signalling.** β-Catenin (β-cat) mediates both growth-factor- and WNT-mediated signalling pathways. The interaction of a WNT-ligand with its transmembrane receptor — 'frizzled receptor' — recruits dishevelled protein, which inactivates glycogen synthase kinase-3β (GSK-3β) by phosphorylation at serine-9. On the other hand, interaction of a growth factor with receptor tyrosine kinase (RTK) leads to the activation of phosphatidylinositol 3-kinase (PI3K), which, in turn, phosphorylates AKT/protein kinase B (PKB). Phosphorylated AKT also inactivates GSK-3β by serine-9 phosphorylation. A tumour-suppressor protein phosphatase and tensin homologue deleted on chromosome 10 (PTEN) blocks AKT-mediated inactivation of GSK-3β. GSK-3β — a component of a multiprotein complex that consists of GSK-3β, adenomatous polyposis coli (APC), axin and conductin — regulates the intracellular fate of β-catenin, which, in its membrane-bound form, acts as a component of the cell–cell adhesion machinery and, in its free cytosolic form, acts as a signalling molecule. In the absence of a growth factor or WNT signal, GSK-3β phosphorylates cytosolic β-catenin at amino-terminal serine (S) and threonine (T) residues, which is then targeted for ubiquitylation (Ub) by ubiquitin ligase followed by proteasomal degradation. In response to the above stimuli, the inactivation of GSK-3β results in cytosolic stabilization of β-catenin. Besides inactivation of GSK-3β, mutation of either APC or axin as well as β-catenin causes its stabilization in the cytoplasm. Stabilized cytosolic β-catenin translocates to the nucleus and binds to T-cell factor (TCF)/lymphoid enhancing factor (LEF). The β-catenin–TCF/LEF complex acts as a transcription factor and activates transcription of genes that are involved in the regulation of cellular growth processes. Some chemopreventive phytochemicals have recently been reported to target β-catenin-mediated signalling pathways. Curcumin downregulates β-catenin through caspase-mediated degradation of the protein, resulting in decreased DNA-promoter-binding activity of the β-catenin–TCF/LEF complex and reduced levels of c-MYC protein. Caffeic acid phenethyl ester (CAPE) and resveratrol also attenuate expression of β-catenin. Epigallocatechin gallate (EGCG) inhibits β-catenin–TCF4 reporter activity and reduces β-catenin protein levels. Indole-3-carbinol shifts the pattern of β-catenin mutations, thereby hampering its nuclear translocation.

As upregulation of COX2 promotes tumorigenesis, and β-catenin is found to regulate *COX2* expression, modulation of β-catenin signalling could be another molecular target for chemoprevention by dietary phytochemicals.

Future directions

Chemoprevention by edible phytochemicals is now considered to be an inexpensive, readily applicable, acceptable and accessible approach to cancer control and management. With healthcare costs being a key issue today, it would be cost-effective to promote the awareness and consumption of phytochemicals as a cancer-preventive strategy for the general public.

Several nutrients and non-nutritive phytochemicals are being evaluated in intervention trials for their potential as cancer chemopreventive agents. Despite significant advances in our understanding of multistage carcinogenesis, little is known about the mechanism of action of most chemopreventive agents. The chemopreventive effects that most dietary phytochemicals exert are likely to be the sum of several distinct mechanisms. Disruption or deregulation of intracellular-signalling cascades often leads to malignant transformation of cells, and it is therefore important to identify the molecules in the signalling network that can be affected by individual chemopreventive phytochemicals to allow for better assessment of their underlying mechanisms.

In many cases, the chemopreventive effects of dietary chemopreventives in cultured cells or tissues are only achievable at supraphysiological concentrations — such concentrations might not be attained when the phytochemicals are administered as part of diet. Furthermore, phenolic phytochemicals are often present as glycosides or are converted to other conjugated forms after absorption, which might further lower the bioavailablity. Both pharmacokinetic properties and bioavailability are key problems in investigating the dietary prevention of cancer and should be assessed carefully before undertaking intervention trials with dietary supplements.

The development and use of chemopreventive agents for intervention trials involve many scientific disciplines. With the advances in techniques to assess single nucleotide polymorphisms (SNPs), we are now more aware of the specific genes that can directly and indirectly contribute to individual differences in the susceptibility to carcinogenesis. When high-risk groups are identified, practitioners might be able to recommend specific dietary supplements that can modulate or restore the cellular-signalling events that are likely to be disrupted in these individuals. The term 'nutragenomics' has been coined, and much attention is being focused on this relatively new area of research. Tailored supplementation with designer foods that consist of chemopreventive phytochemicals — each having their own distinct anticancer mechanisms — will be available in the near future. These should be developed in line with advances in the genetic and molecular epidemiology of carcinogenesis.

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Acknowledgements
The author thanks the members of his laboratory, especially H.K. Na, J.K. Kundu, K.S. Chun, J.S. Lee, M.H. Chung, E. Kim and J.M. Lee (currently at the University of Wisconsin-Madison) for having prepared the table and illustrations, as well as sorting out the references. Work in the author's laboratory is supported by research grants from the Korea Institute of Science and Technology Evaluation and Planning (KISTEP) for functional food research and development, Ministry of Science and Technology.

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