MINIREVIEW

Isoprenoid-Mediated Inhibition of Mevalonate Synthesis: Potential Application to Cancer (44413)

CHARLES E. ELSON,*,[1] DENNIS M. PEFFLEY,† PATRICIA HENTOSH,† AND HUANBIAO MO*

Department of Nutritional Sciences, College of Agricultural and Life Sciences, University of Wisconsin-Madison, Madison, Wisconsin 53706; and Department of Cellular and Molecular Pharmacology,† Finch University of Health Sciences/The Chicago Medical School, North Chicago, Illinois 60064–3095*

Abstract. **Pure and mixed isoprenoid end products of plant mevalonate metabolism trigger actions that suppress 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase activity. These actions modulate HMG CoA reductase mRNA translation and the proteolytic degradation of HMG CoA reductase. Such post-transcriptional events, we propose, are activated directly by acyclic isoprenoids and indirectly by cyclic isoprenoids. Isoprenoids, acting secondarily to the dominant transcriptional effector of sterologenesis, modestly lower cholesterol levels, if and only if, sterologenesis is not repressed by a saturating imput of dietary cholesterol. An anomaly associated with tumor growth—a sterol feedback–resistant HMG CoA reductase activity—ensures a pool of sterologenic pathway intermediates. Such intermediates provide lipophilic anchors essential for membrane attachment and biological activity of growth hormone receptors, nuclear lamins A and B, and oncogenic *ras*. Tumor HMG CoA reductase retains high sensitivity to the isoprenoid-mediated secondary regulation. Repression of mevalonate synthesis by plant-derived isoprenoids reduces *ras* and lamin B processing, arrests cells in G1, and initiates cellular apoptosis. This unique tumor cell–specific sensitivity allows isoprenoids to be used for tumor therapy, an application emulating that of the statins, but one free of adverse effects. When evaluated at levels provided by a typical diet, isoprenoids individually have no impact on cholesterol synthesis and tumor growth. Nonetheless, isoprenoid-mediated activities are additive, and, sometimes synergistic. Therefore, the combined actions of the estimated 23,000 isoprenoid constituents of plant materials, acting in concert with other chemopreventive phytochemicals, may explain the lowered cancer risk associated with a diet rich in plant products. In contrast, that lowering of cancer risk does not correspond to supplemental intake of other dietary factors associated with fruits, vegetables, and cereal grains, namely fiber, β-carotene, vitamin C, and vitamin E, and only weakly to supplemental folate.** [P.S.E.B.M. 1999, Vol 221]

Adherence to dietary guidelines (1, 2) that emphasize the importance of fruits, vegetables, and grains (3–5) could yield an estimated 35% reduction in cancer deaths (6–9). Fruits, vegetables, and grains offer a pharmacopia of potential anticarcinogenic agents (10). Included among these agents are constituents deemed to play roles in health maintenance, specifically the nondigestible carbohydrates and a group of micronutrients consisting of β-carotene, ascorbic acid, α-tocopherol, and folic acid. Results of trials that tested the chemopreventive actions of these constituents (11–17) and meta-analyses of the epidemiologic data (18) are, at best, inconclusive. Also included in the pharmacopia is an array of non-nutritive phytochemicals with demonstrated potential as anticarcinogenic agents. Inhibitors of the carcinogenic process may be broadly classified either as blocking or suppressing agents (19–21). Blocking agents act at the initiation phase of chemical car-

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[1] To whom requests for reprints should be addressed at Department of Nutritional Sciences, University of Wisconsin-Madison, 1415 Linden Drive, Madison, WI 53706. E-mail: elson@nutrisci.wisc.edu

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cinogenesis by preventing the formation of carcinogens from precursor substances and by preventing carcinogenic agents from reaching or reacting with critical target sites. Blocking agents include fiber, the antioxidant nutrients, the dithiolthiones, glucosinolates and indoles, isothiocyanates, flavonoids and phenols. Blocking actions include the induction of Phase I and II detoxifying enzymes, the dilution or binding of carcinogens in the gastrointestinal tract, and the capture of free radicals. Suppressing agents repress the expression of neoplasia in initiated cells. Suppressing agents include the protease inhibitors, phytosterols, and allium compounds. These agents might provide substrates for the formation of antineoplastic agents or alter hormone status. Blocking and suppressing agents may have complementary, and occasionally overlapping, mechanisms of action (22).

More recently, the blocking and suppressing activities conferred by secondary products of plant mevalonate metabolism have been recognized (23). The estimated 23,000 secondary products of plant mevalonate metabolism, differing in size, complexity, and function, are hereafter termed isoprenoids. Some are "pure" isoprenoids of varying structural complexity but consisting only of multiples of the five-carbon isoprene unit (x), [e.g., monoterpenes (2x), sesquiterpenes (3x), diterpenes (4x), triterpenes (6x), tetraterpenes (8x), and polyterpenes (nx)] (24–26). Others are "mixed" isoprenoids: the prenylated coumarins, flavones, flavanols, isoflavones, chalcones, quinones, and chromanols, each with only a part of the molecule being derived *via* the mevalonate pathway (27).

Our studies of the antitumorigenic properties of dietary isoprenoids evolved from the anomalous finding that, contrary to the activation of cholesterol synthesis obtained by administering bile acid sequestrants to animals fed cholesterol-free diets, cholesterol synthesis was suppressed in livers of animals fed fiber-rich grains. This anomaly was resolved with the finding that a group of mixed isoprenoids extracted from the barely bran fraction, the tocotrienols, suppressed cholesterol synthesis (28–30). When depleted of the tocotrienols by solvent extraction, the bran fraction had no impact on cholesterol metabolism.

At that time certain aspects of cholesterol metabolism appeared to be relevant to cancer. The characteristically elevated and feedback-resistant sterologenic activity described in the following sections enabled rapidly proliferating tissues (e.g., embryonic tissues, regenerating liver, and tumors) to synthesize and accumulate cholesterol at much higher rates than those observed in nonproliferating tissues. John Elegbede, a graduate student in this laboratory, working also with University of Wisconsin colleague Milton Yatvin, sought to modulate cholesterol metabolism in transplanted CA755 mammary tumors by feeding host mice a diet supplemented with a crude barley extract. Independent of an effect on cholesterol content, the growth of the transplanted mammary tumors was substantially retarded by the barley extract (31). Since α-tocotrienol, the cholesterol-suppressive metabolite in the barley extract (28), was not available through commercial channels we, working with University of Wisconsin colleague Michael Gould, evaluated the impact of two commercially available end products of plant mevalonate metabolism similarly reported to suppress mevalonate synthesis, *d*-limonene (32) and abscisic acid (33), on chemical carcinogenesis. We and others report findings that these and other pure and mixed isoprenoids suppress chemically initiated carcinogenesis (reviewed in Refs. 34–37); Elegbede *et al.* reported the unanticipated finding that a diet augmented with 10% *d*-limonene (735 mmol/kg diet) caused the regression of chemically initiated primary mammary tumors (38). Diets supplemented with 2% perillyl alcohol (130 mmol/kg) were later shown to cause tumor regression (39).

Building on our recent review of the responses of diverse tumor cell lines to pure and mixed isoprenoids (37), we add HL-60 promyelocytic (40) and NB4 (41) leukemic cells, MD592 IL-3 dependent myeloid cells (41), C-4-1 cervical carcinoma cells (42), DU-145 prostate carcinoma cells (42), CEM-C1 leukemic (43) cells, HepG2 cells (44), Caco-2 colon adenocarcinoma cells (40), A549 lung adenocarcinoma cells (45), Ishikawa endometrial cancer cells (46), NCI-H226 squamous lung cancer cells (46), and MIA PaCa2 pancreatic tumor cells (47) to the list of human tumor cell lines shown to respond to the growth-suppressive action of isoprenoids. On the other hand, human CCD-18co colon fibroblasts (40), CF-3 fibroblasts (42, 48), normal protate cells (42), and foreskin fibroblasts (46, 48) as well as hamster pancreatic ductal (49) and porcine aortic (42) epithelial cells are relatively resistant to isoprenoid-mediated suppression of cell growth.

Consistent with this observation of a differential sensitivity between normal and neoplastic cells to the growth suppressive action of isoprenoids (40–49) are numerous animal studies showing that isoprenoids have no impact on the growth of animals treated with diverse carcinogens (reviewed in Refs. 35, 36). Protocols differing in type of carcinogen administered and feeding regimen offer substantial documentation that isoprenoids suppress both the initiation and promotion/progression stages of chemically initiated carcinogensis. Carcinogenesis models typically use heroic chemoprotective doses (35, 36). However, dietary isoprenoids suppress the growth of implanted tumors when fed at considerably lower doses (34–37).

The tumor implant model permits evaluation of the impact isoprenoids have on the growth of established tumors independent of a confounding effect on the initiation stage of tumor development. Monoterpenes suppressed the growth of implanted B16F10 melanomas, P388 leukemic cells, Morris 7777 hepatomas, PC-1 pancreatic adenocarcinomas, and colon CT-26 tumors. The growth of B16F10 melanomas (50) and P388 leukemia cells (51) implanted in mice, Morris 7777 hepatomas implanted in buffalo rats (50), and PC-1 pancreatic adenocarcinomas implanted in Syrian Golden hamsters (47) was suppressed by the acyclic monoterpene, geraniol (6.5, 6.5, 23, and 130 mmol/kg diet re-

spectively). The cyclic monoterpenes, *d*-limonene (360 mmol/kg diet) and perillyl alcohol (260 mmol/kg diet), respectively suppressed metastatic growth of murine colon CT-26 tumors (52) and PC-1 pancreatic adenocarcinomas (47). The growth of implanted PC-1 pancreatic adenocarcinomas was suppressed by the acyclic sesquiterpenoid farnesol (90 mmol/kg diet) (47) and that of the B16 melanoma, by the cyclic sesquiterpenoid, β-ionone (2 mmol/kg diet) (53), a potent suppressor of chemically initiated mammary carcinogenesis (54). The mixed isoprenoid, *d*-γ-tocotrienol suppressed growth of B16 melanomas when substituted for *dl*-α-tocopherol in the standard AIN 766A dietary formulation (53), a level approaching 0.005% (116 μmol/kg) of the diet.

In the following section we present the hypothesis that dysregulation of sterologenesis characteristic of tumors traces to an over-expressed sterol feedback resistant 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase activity that retains sensitivity to isoprenoid-modulated downregulation. We first review mechanisms underlying the regulation of HMG CoA reductase in sterologenic tissues and its dysregulation in tumor tissues.

In animal cells, a finely tuned metabolic feedback mechanism involving transcriptional and post-transcriptional inputs maintains a pool of mevalonate pathway intermediates essential for cell survival in addition to maintaining cholesterol homeostasis (55). Cholesterol, the bulk end product of the pathway in sterologenic tissues, exerts transcriptional control on sequential activities in the mevalonate pathway, principally those catalyzed by HMG CoA synthetase, HMG CoA reductase and farnesyl pyrophosphate synthetase. HMG CoA reductase is the rate-limiting enzyme of mevalonate biosynthesis as well as the most highly regulated enzyme in this series (55–57). The multivalent regulation of HMG CoA reductase (56) integrates activities at the transcriptional (55–70), post-transcriptional (55, 59–64, 71–74), and post-translational (55, 61, 71–83) levels. Sterol-mediated regulation of transcription, the dominant regulatory site in sterologenic tissues, is mediated through the sterol regulatory element, (SRE), a promoter-enhancer octanucleotide sequence, GTGCGGTG, located in the 5′ flanking region of the reductase gene (55, 66, 68). The binding of transcription factors Red 25 (84) and SRE binding protein-1 (SREBP-1) (85) within the SRE region is required for sterol feedback–mediated downregulation of HMG-CoA reductase transcription (68, 84, 86). The post-translational control of HMG CoA reductase activity, a secondary level of control called into play when cellular isoprenoid requirements are satisfied, is mediated by a nonlysosomal cysteine protease (81–83). This protease activity, a process accelerated by sterols and a mevalonate-derived nonsterol product (60–63, 74–82), has high specificity for the 97-kDA HMG CoA reductase with intact hydrophobic domains in the endoplasmic reticulum. If membrane domains are deleted, neither sterols nor the mevalonate-derived nonsterol product affects reductase turnover (79). Current studies support the hypothesis that farnesol is the mevalonate-derived nonsterol mediator of HMG CoA reductase degradation (81, 82). Supporting this concept is the detection (87) and characterization of hepatic microsomal allyl pyrophosphate pyrophosphatase activities (88), one with high specificity for farnesyl pyrophosphate (81, 88, 89). Modulation of this activity may play a critical role (81) in elevating cellular farnesol, because, within a physiological range (5–50 μ*M*), farnesol, and with diminishing potency farnesal and farnesoic acid, activates the orphan nuclear receptor FXRα (90, 91). Farnesol, a sesquiterpene, has a short biological half-life due to alcohol oxidoreductase activities localized in mitochondrial and peroxisomal fractions (89) that catalyze its conversion to farnesoic acid, a prenoic acid with low affinity for the orphan receptor (90). Microsomal cytochrome P-450-dependent monooxygenase activities catalyze the conversion of farnesoic acid to 12- and 15-carbon α, ω-dibasic prenyl acids (87, 89, 92–97) that are excreted through the kidney (Fig. 1).

Recent studies demonstrate that differences in HMG CoA reductase activity and protein levels cannot be attributed solely to a change in HMG CoA reductase mRNA level or to the degradation of membrane-bound enzyme. These studies suggest that translational control of HMG CoA reductase synthesis has an equally important role in regulating reductase activity in animal cells (59–63, 71–74, 98). The regulation of translation occurs at the level of both initiation and elongation of protein synthesis, and all reported mechanisms depend on specific sequences or secondary structural features of RNA transcripts, especially those associated with both 5′-untranslated leader (5-UTL) (99) and 3′-untranslated (3′-UT) (100) sequences. In addition, protein interactions with such sequences have an important process in this translational regulation (99, 100). Translational control of HMG CoA reductase was initially described in a mevalonate auxotroph, the CHO fibroblast Mev-1 (60), and in Syrian hamster C-100 cells incubated with lovastatin, a competitive inhibitor of mevalonate synthesis (61, 69, 76). A study by Peffley and Sinenski (60) demonstrated that a mevalonate-derived nonsterol product and an oxysterol act synergistically with lovastatin to achieve a greater suppression in the rate of reductase synthesis than can be attributed to their individual effects on reductase mRNA. The discrepancy between large changes in enzyme activity and rates of synthesis, independent of a change in HMG CoA reductase mRNA level, points to a translational control mechanism, one that is mediated by a nonsterol mevalonate-derived product of the mevalonate pathway. We found that a mevalonate-derived nonsterol product distal to farnesyl diphosphate and proximal to lanosterol in the sterol pathway (59) suppresses HMG CoA reductase synthesis by reducing the efficiency of HMG CoA reductase mRNA translation (59, 101). Candidate regulatory signal molecules include squalene, farnesyl pyrophosphate or a derivative (farnesol), or farnesyl-pyrophosphate-generated inorganic pyrophosphate (102). Our finding that an intact 5′-UTL region of reductase

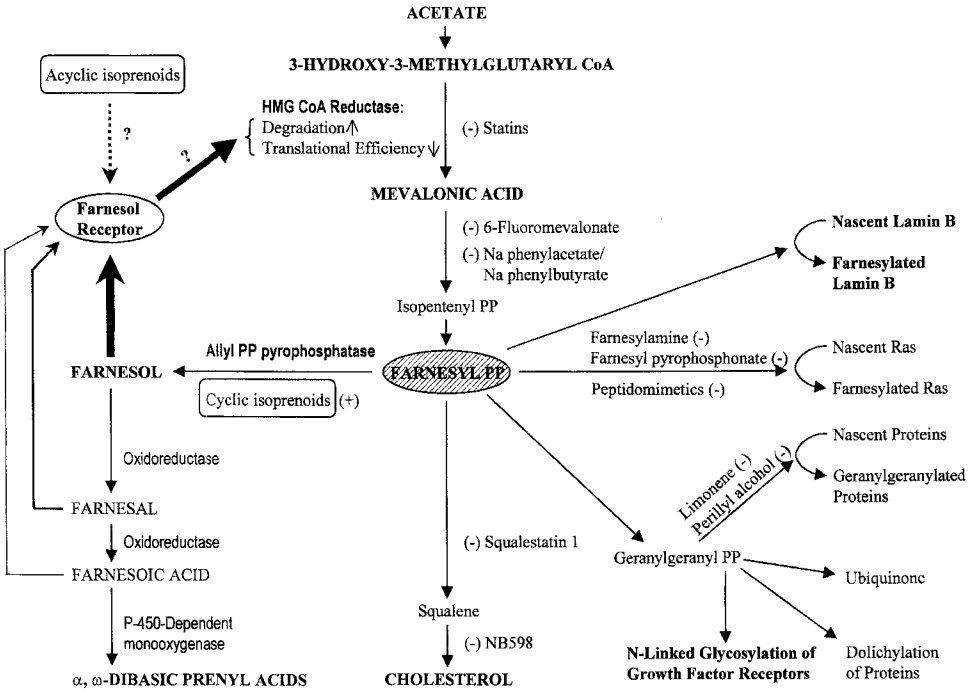

Figure 1. An orientation to the mevalonate pathway with delineation of prospective role of farnesol as a secondary regulator of mevalonate synthesis, farnesol oxidation, nonsterol products essential for cell survival, sites for pharmacological intervention, and prospective sites of isoprenoid-mediated actions.

mRNA is required for this regulatory action (59) indicates that it occurs at the level of initiation. Our recent studies (103) suggest that a GC rich stem-loop structure in the 5′-UTL of class I reductase transcripts has a role in mediating reductase mRNA translational efficiency. Another type of reductase post-transcriptional control, the oxysterol-mediated degradation of HMG CoA reductase mRNA that functions independently of oxysterol-mediated transcriptional control, has also been reported (64, 104). This degradation requires the presence of at least 1600 bases in the 3′-untranslated (UT) sequence of HMG CoA reductase mRNA (104). The putative AUUA instability elements in the 3′-UT region of HMG CoA reductase mRNA may be required for this regulatory process. In summation, farnesol appears to be a key player in the post-translational control of reductase by inducing an HMG CoA reductase-specific protease (81, 82). Farnesol, or another isoprenoid distal to farnesol (59), may also mediate the translational control of HMG CoA reductase synthesis (81, 82).

In 1958 Potter (105) advanced the deletion hypothesis, namely that a fundamental lesion of malignant cells is a feedback control defect that allows the uninhibited synthesis of key growth intermediate. Shortly thereafter, Siperstein and Fagan (106) reported the dysregulation of cholesterol synthesis in tumors and tumor-bearing animals. Subsequently, the uncoupling of HMG CoA reductase activity from sterol-mediated feedback regulation was shown to occur in diverse tumors as well as in embryonic and differentiating tissues and in carcinogen-treated and regenerating liver (107–115). George and Goldfarb (108) reported that the elevated sterol feedback-resistant HMG CoA reductase activity in tumors retains sensitivity to feedback regulation by mevalonate; as delineated above, farnesol appears to be the mevalonate-derived nonsterol feedback mediator of both the translation of HMG CoA reductase mRNA (59, 101) and HMG CoA reductase degradation (81, 82). A second study detailing the anomalies in tumor HMG CoA reductase activity is depicted in a report by Bennis *et al.* (115). Maximally induced and sterol-suppressed HMG CoA reductase activities in A549 human lung adenocarcinoma cells were two- and four-fold respectively those present in normal fibroblasts. However, the sterol feedback–resistant HMG CoA reductase activity in the adenocarcinoma retained sensitivity to the post-transcriptional control. It has been that an aberrant pattern of DNA methylation on the HMG CoA reductase gene might hinder sterol feedback regulation (116, 117).

Building on the analogy that the mevalonate pathway characteristic of tumors bears striking similarity with that operative in fungi (118), yeast (119–125), protozoa (126), parasitic protozoa (126–128), helminths (126), and insects (129, 130), we add the report that translation of the mRNA coding the dominant of the two sterol feedback–insensitive forms of HMG CoA reductase operative in logarithmically growing yeast, Hmg 1p, is sensitive to nonsterol-mediated feedback regulation (123). In contrast, the lesser reductase activity associated with the endoplasmic reticulum, Hmg2p, is sensitive to degradation by a nonsterol-triggered protease activity (119). In proliferating yeast cells, Hmg1p activity is induced and concentrated in the nuclear envelope, whereas Hmg2p activity, prominantly under low growth conditions, is concentrated in the peripheral endoplasmic reticulum

(124, 125). Recent findings show that mammalian cells similarly express two isozymes of HMG CoA reductase, one of 97 kDa in the endoplasmic reticulum (131) and a second of 90 kDa in peroxisomes (132). Findings that the low-speed pellet fractionation of maximally lysed mucosal cells retains a substantial proportion (60%) of cellular HMG CoA reductase activity (133) suggests that the envelope of nuclei of proliferating mammalian cells has an associated endoplasmic reticulum.

The dysregulation of HMG CoA reductase activity was deemed to be a consequence of excessive demand for cholesterol in the assembly of membranes of rapidly growing tumors. Contrary to our assumption that cholesterol was the rate-limiting substrate for tumor growth, we found cholesterol-enriched membranes in tumors and cholesterol-depleted membranes in livers of host mice that were fed the cholesterol-suppressive (28) barley extract (31). The seminal report of post-translational incorporation of a mevalonic acid–derived metabolic product into animal proteins (134) suggested an alternative rationale for explaining the impact of cholesterol-suppressive isoprenoids on tumor growth. Investigators first identified the carboxyterminal cysteine residue of lamin B (135–137) and immediately thereafter that of p21*ras* (138–140) as the site where the 15-carbon mevalonic acid–derived farnesyl group (140, 141) is covalently attached. Prenylated proteins in animal cells fall into four size classes, 41–46 kDa, 53–55 kDa, 66–72 kDa, and 21–28 kDa (135–146). The CaaX carboxy-terminal sequence motifs of the latter two classes direct, in sequence, the transfer of the farnesyl moiety from farnesyl pyrophosphate to the cysteine sulfhydryl, the proteolysis of the terminal xxA amino acids, and carboxymethylation of the COOH-terminal farnesylated cysteine (144, 146). This processing is essential for the biological function of the nuclear lamins (147–150) and virtually all members of the *ras* superfamily of proteins (138, 140, 145, 146).

The post-translational processing of *ras,* mutated forms of which are present with high frequency in various human tumors, offers an opportunity for chemotherapeutic intervention. One pharmacological strategy is to inhibit the farnesyl-protein transferase activity (Fig. 1). This approach has resulted in the development of nonhydrolyzable farnesyl pyrophosphate analogs, e.g., farensylamine, farnesyl pyrophosphonate (151), and peptidomimetics of the *ras* carboxy-terminal CaaX motif (151–153). During the course of our studies (reviewed in Refs. 34–37) we discovered that *d*-limonene, a cyclic monoterpene, decreased with some selectivity, the incorporation of radiolabeled mevalonolactone into 21–26-kDa proteins (154). Perillic acid, dihydroperillic acid, and limonene-1,2-diol appear in the plasma of humans following the administration of *d*-limonene (155). These products and perillyl alcohol products suppress, with greater potency than *d*-limonene, protein isoprenylation and cell proliferation (155–158). *d*-Limonene metabolites may have chemotherapeutic potential since farnesylation-dependent membrane localization is critical for the growth-promoting function of mutated *ras*. Other studies showed that perillyl alcohol lowered the level of farnesylated *ras,* not by inhibiting farnesyl-protein transferase, but by lowering cellular *ras* levels either by suppressing *ras* synthesis or by increasing *ras* degradation (159). Although the concentration of perillyl alcohol required to supress cellular levels of *ras* in the laboratory (159) is orders of magnitude higher than the level most likely achievable after pharmacological dosing (157), this monoterpene, alone among the putative prenyl-protein transferase inhibitors, is undergoing Phase II clinical evaluation (160); a pilot study combining *d*-limonene and lovastatin yielded objective clinical responses in six of nine patients (161).

The second pharmacological strategy is to suppress synthesis of farnesyl pyrophosphate by inhibiting synthesis of its sequential precursors, mevalonic acid and isopentenyl pyrophosphate (Fig. 1). The discovery of compactin, a competitive inhibitor of HMG CoA reductase, the enzymic activity considered to be the rate-limiting step in mevalonate biosynthesis, led to the recognition that a mevalonate-derived substance other than cholesterol is required for cell growth (162). Cells incubated with compactin and related substances (statins, Fig. 1) are arrested at the G1/S interface of the cell cycle (163–165); this arrest is reversed with mevalonate but not with cholesterol supplements (163–167). Among the regulation-related events occurring prior to the onset of the S phase is the enhanced synthesis of HMG CoA reductase (165), which is resistant to sterol feedback regulation (168). Asynchronous (167) and synchronous (163–165) cells arrested at the G1/S interface of the cell cycle exhibit an altered morphology.

The statin-mediated suppression of mevalonate synthesis (Fig. 1) suppresses *ras* farnesylation as well as the isoprenylation of other proteins localized in the plasma membrane, endoplasmic reticulum, Golgi membrane, nuclear lamina, mitochondrial membranes, and cytosol (57). Lovastatin is widely used to treat hypercholesterolemia. A five-year safety and efficacy study composed of more than 700 patients showed the drug (<2 mg/kg body weight) to have a good medium-term safety profile. Within this population cancer deaths were reduced by 33% (169). In a Phase I study, 88 patients with solid tumors were aggressively treated monthly with 7-day course of lovastatin (2–45 mg/kg body weight). Plasma drug concentrations reached 3.9 μM, a concentration associated with *in vitro* antiproliferative activity. Rhabdomyolysis, nausea, diarrhea, and fatigue, the dose-limiting toxicities, appeared with doses in excess of 25 mg/kg body weight; some patients with refractory primary CNS tumors given 30 mg lovastatin/kg body weight showed minor responses. Prophylactic administration of ubiquinone (Fig. 1) prevented the development of rhabdomyolysis (170).

As a consequence of inhibiting mevalonate pyrophosphate decarboxylase activity sodium phenylacetate, sodium phenylbutyrate and 6-fluoromevalonate suppress the synthesis of isopentenyl pyrophosphate, a precursor of farnesyl

pyrophosphate (Fig. 1). Phase I clinical and pharmacologic evaluations employing continuous iv infusion of sodium phenylacetate (300 mg/kg body weight) produced steady state plasma levels on the order of 0.2-0.4 m*M*; refractory Hodgkin's disease responded to an average plasma phenylbutyrate concentration falling in this range (171–174). A prostate tumor and a malignant glioma responded to phenylacetate infused twice daily with 150 mg/kg body weight (175, 176). The proliferation of a variety of leukemia- and lymphoma-derived (177) and arterial smooth muscle (178) cells was suppressed by 6-fluoromevalonate. The statins (138), phenylacetate (179), and fluoromevalonate (138, 170) suppress farnesyl pyrophosphate synthesis and concomitantly, the farnesylation and membrane association of *ras*. Although inhibitors targeted to mevalonate pathway at sites distal to farnesyl pyrophosphate, that is squalene synthase (Squalestatin 1) and squalene epoxidase (NB598), block cholesterol biosynthesis (Fig. 1), they have little impact on cell proliferation (178).

Suppression of mutated *ras* farnesylation does not fully explain the tumor-suppressive action of inhibitors targeted to the mevalonate pathway. The growth of tumor cells with wild-type as well as mutated *ras* is suppressed by statins (180–184). The impact of statins on cell growth may be better explained by findings that competitive inhibitors of HMG CoA reductase activity arrest cells at the G1/S interface of the cell cycle (163–166). Mevalonate synthesis (Fig. 1) is essential for protein dolichylation (185) and the dolichol-supported N-linked glycosylation and membrane attachment of growth factor receptors (186). Also critical to explaining their impact on cell growth are demonstrations that statins initiate p53-independent apoptosis across a spectrum of tumor cell lines (184, 187–194).

A sterol feedback–resistant HMG CoA reductase activity induced during the G1 phase of the cell cycle (165, 168) ensures a pool of farnesyl pyrophosphate, the rate-limiting substrate for the post-translational modification of the carboxyl-terminal cysteine in the CaaX sequence of neosynthesized prelamin A and lamin B (145, 149, 150, 195, 196). This covalent modification is essential to the nuclear localization of the lamins, the structural and functional integrity of the nuclear lamina, S-phase DNA synthesis, and the formation of nuclear envelopes in daughter cells following completion of mitosis (147, 149). During mitosis, a peptide consisting of 18 amino acids including the farnesylated carboxyterminal cysteine is cleaved from prelamin A. The soluble lamin A subunit is dispersed through the cell whereas lamin B remains associated with remnants of the nuclear membrane (195). Reassembly of the daughter chromosomes is blocked by lovastatin. The resulting breakdown of the nuclear membrane due to the inhibition of nuclear lamin processing renders DNA accessible to p53-independent apoptotic endonuclease activities lacking specificity for internucleosomal linker DNA sections (187, 188, 196–198).

Because the aforementioned post-translational processing is essential to the biological functions of cell growth–associated proteins, it presents opportunities for developing novel agents for cancer chemotherapy. Targeting these agents to cancer cells presents a challenge as farnesylation, and more so geranylgeranylation, is essential to the biological function of many proteins with activities associated with cell growth (141–145). Interference with these biological functions underlies the dose-limiting toxicities of the statins.

The finding that material extracted from barley bran suppressed cholesterol synthesis (28) was resolved with the demonstration that tocotrienols suppress HMG CoA reductase activity (28) by two post-transcriptional actions, the degradation of HMG CoA reductase by a nonlysosomal protease and a decrease in the efficiency of HMG CoA reductase mRNA translation (29). The tocotrienols may be viewed as homologs of farnesol, a view reflecting their shared impact on HMG CoA reductase activity. Within the concentration range of 5–50 μM, farnesol (78) and the farnesyl derivatives, farnesyl acetate and ethyl farnesyl ether (199) accelerate degradation of HMG CoA reductase. γ-Tocotrienol, a more potent farnesyl homolog acting within the range of 1–4 μM, accelerates HMG CoA reductase degradation and decreases the efficiency of HMG CoA mRNA translation (29). The difference in the reductase-suppressive potencies of farnesol and γ-tocotrienol is, we suggest, due to the mixed isoprenoid's resistance to the aforementioned conversion by cytosolic and microsomal activities. The geranylated tocol analog similarly suppresses HMG CoA reductase activity but with lower potency than the farnesylated homologs (200, 201). It remains to be determined whether the analogs are ligands for the orphan nuclear receptor. At the upper limit tested (50 μM) geraniol neither activated the receptor (202) nor accelerated HMG CoA reductase degradation (78); however, geraniol (203), citral (203), lycopene (204), and the mixed acyclic isoprenoid, ubiquinone (205), are reported to suppress HMG CoA reductase activity though with lower potency than farnesol. The saturated tocols, the tocopherols, had no impact on HMG CoA reductase activity (28, 29); much later we determined that the tocopherols attenuated the impact of dietary tocotrienol (206) presumably by interfering with the latter's absorption, transport, and tissue deposition (207).

Cyclic isoprenoids similarly suppress HMG CoA reductase activity. Among these, menthol (32), β-carotene (208), and β-ionone (54) are reported to lower HMG CoA reductase mass. The cyclic isoprenoid effect may be secondary to microsomal farnesyl pyrophosphate pyrophosphatase–and perhaps, microsomal and peroxisomal farnesol pyrophosphate kinase–mediated changes in cellular farnesol content (81, 87–89) as we have shown that cyclic isoprenoids induce an allyl pyrophosphate pyrophosphatase activity (209).

Investigations of the farnesol-mediated modulation of HMG CoA reductase activity have focused on reductase degradation (78, 81–83). Cyclic (32) and mixed acyclic (29)

isoprenoids trigger reductase degradation either by elevating cellular farnesol or by acting as farnesol homologs. Receiving less notice was the finding that the tocotrienols suppress the efficiency of HMG CoA reductase mRNA processing in HepG2 cells (29). We have preliminary findings showing that perillyl alcohol, geraniol, and β-ionone similarly suppressed reductase mRNA processing in HL-60 cells (210). In follow-up studies, HL-60, K562, Caco-2, and MCF-7 cells were incubated with 100 μ*M* β-ionone. Incorporation of radiolabeled methionine into HMG CoA reductase by the respective cell lines, corrected for changes in reductase mRNA levels, was suppressed by 90, 90, 40 and 60%. We earlier delineated our finding that an endogenous mevalonate-derived signal molecule, presumably farnesol, suppresses the efficiency of HMG CoA reductase mRNA translation (59, 101). Our preliminary showing of an isoprenoid-mediated downregulation of reductase mRNA processing is, we feel, consistent with isoprenoid-mediated changes in cellular farnesol.

A number of studies demonstrate the cholesterol-lowering action of pure and mixed isoprenoids in experimental animals (reviewed in Refs. 34–37). We have evaluated the impact of citral (211) and γ-tocotrienol (212) on cholesterol levels of hypercholesterolemic human subjects. In the first study, a group of 22 hypercholesterolemic subjects (315 ± 39 mg cholesterol/dl) was treated with 140 mg lemongrass oil (citral) daily for 90 days. The paired difference in cholesterol (−15 ± 30 mg/dl) approached significance. An in-depth analysis of the individual responses revealed a subset of responders (−38 ± 40 mg/dl, $P < 0.025$) and a subset of resistors (−1 ± 24 mg/dl). On release from the study, the cholesterol levels of subjects who responded to citral returned to pretest levels (211). In the second, a group of 16 hypercholesterolemic subjects was adapted to the American Heart Association Step 1 Diet for 8 weeks. During that period, the mean cholesterol value fell from 7.29 to 6.69 m*M*. The subjects then received 140 mg γ-tocotrienol/day for 4 weeks. The treatment effected an additional 0.87 ± 0.10 m*M* decrease ($P < 0.001$) in cholesterol (212).

During the course of these studies, we recognized that the potency with which an isoprenoid suppresses hepatic HMG CoA reductase activity predicts its potency as an antitumor agent (35, 36, 213). The sensitivity of HMG CoA reductase activity in tumor tissues to isoprenoid-mediated downregulation is several-fold greater than that of reductase activity in sterologenic tissues. For example, Parker *et al.* (29) determined that elevated HMG CoA reductase activity in HepG2 hepatoma cells was about 40-fold more sensitive than reductase activity in primary hepatocytes to tocotrienol-mediated downregulation. This, we believe, explains the very marked impact isoprenoids have on tumor growth, the very modest impact isoprenoids have on serum cholesterol levels, and the absence of an isoprenoid impact on the growth of animals.

The B16(F10) melanoma provides a rigorous model for assessing, *in vitro* (40, 51, 214–218) and *in vivo* (50, 53, 218, 219) antitumor potency of lovastatin (218) and other agents. We have used this cell line for screening tumor growth–suppressive potencies of lovastatin (53) and assorted isoprenoids. The IC_{50} value is the concentration of an isoprenoid that inhibits by 50% the net increase in cell count at a time point within the linear growth period plotted for control B16 melanoma cells. The isoprenoids listed in Table I are grouped according to structural characteristics and potency. IC_{50} values differ by more than 100-fold. A comparison of IC_{50} values determined for the B16 melanoma (53) with those reported for assorted human-derived tumor cell models (40, 45, 47, 156, 220, 221) shows that the latter tend to have greater sensitivity than the murine melanoma to the isoprenoid action (Table II). The relative potencies of isoprenoids determined in *in vitro* (Table I) generally reflects their potencies determined *in vivo* with transplant (47, 50, 52, 53, 220) and chemical carcinogenesis models (23, 31, 54, 222–230).

Based on the relative potencies (Table I), we have constructed and tested the growth-suppressive potencies of blends of isoprenoids (37, 40, 53). The growth-suppressive action of a blend of two cyclic isoprenoids approaches the additive impact predicted for the individual components. A blend of two acyclic isoprenoids, γ-tocotrienol and geranyl tiglate effects a less than predicted impact; this we believe, suggests a competition between the more potent tocotrienol and less potent geranyl tiglate for a common binding site on the farnesol receptor. The important consideration is our finding that synergy is produced by a blend of cyclic and acyclic isoprenoids, that is, the impact of the blend far exceeds the impact predicted by the summation of the impacts of the individual agents (37, 40, 53).

Synergy, we propose, is attained through the distinct actions of the acyclic and cyclic isoprenoids. The acyclic pure (geraniol, farnesol) and mixed (tocotrienols, geranyl derivatives, farnesyl derivatives) isoprenoids trigger the degradation of HMG CoA reductase (81, 82). The cyclic isoprenoids, we propose (209), trigger the allyl pyrophosphate pyrophosphase-mediated diversion of farnesol from the mevalonate pathway (81, 88, 89). Indirect support for this concept comes from reports that cyclic monoterpenes, at concentrations double their IC_{50} values, modestly suppress mevalonate-supported *ras* farnesylation (154, 156, 231). Those reports attributed the monoterpene action to the inhibition of farnesyl protein transferase activity. Subsequent studies showed that perillyl alcohol had little impact on mevalonate-supported farnesyl protein transferase activity (158, 232). This cyclic monoterpene did inhibit mevalonate-supported geranylgeranyl protein transferase types I and II activities (Fig. 1); 50% inhibitions of the respective activities were obtained with 1000 and 500 μ*M* perillyl alcohol (158). The cyclic monoterpene action, we feel (35), is better attributed to the aforementioned allyl pyrophos-

Table I. IC_{50} Values[a] for the Suppression of the Proliferation of B16(F10) Melanoma Cells by Cyclic and Acyclic Isoprenoids and Their Derivatives

Class	IC_{50} (μM)			
	0–100	100–300	300–600	>600
Cyclic				
Pure		perillaldehyde[b] carvacrol[b] thymol[b] anethole[d] β-ionone[b] perillyl alcohol[b] eugenol[c] α-ionone[c]	*d*-limonene[b] α-pinene[c] α-terpineol[c]	verbenone[c] *p*-cymene[c]
β-Ionone derivatives	ethinylcarbinol[e] allylcarbinol[e] phenylcarbinol[e] methylcarbinol[e] benzylcarbinol[e] methylether[e]	ethylcarbinol[e] propylcarbinol[e]		
Acyclic				
Pure	farnesol[b] citral[c]	geraniol[b] nerolidol[c]	linalool[c] citronellol[c] citronellal[c] geranic acid[c] nerol[c]	farnesene[c] citronellic acid[c] myrcene[c]
Derivatives	geranyl anthranilate[f] geranyl tiglate[f] geranyl benzoate[f] farnesyl anthranilate[f] farnesyl tiglate[f] farnesyl benzoate[f] geranyl isovalerate[f] geranyl phenylacetate[f] citral diethyl acetal[f] citral dimethyl acetal[f] citral acetal[f] citronellyl tiglate[f]	geranyl propionate[f] geranyl isobutyrate[f] geranyl formate[f] geranyl acetone[f] geranyl acetate[f] geranyl butyrate[f] geranyl caprylate[f]	neryl acetone[f] neryl acetate[f] citronellyl propionate[f]	
Mixed	*d*-γ-tocotrienol[b] *d*-δ-tocotrienol[b]	*d*-α-tocotrienol[b]		

[a] The IC_{50} value is the concentration of an isoprenoid required to suppress the net increase in tumor cell population by 50%. Isoprenoid effects were determined at midpoints on the linear growth curve determined for control cells.
[b] He *et al.* (53)
[c] Mo and Elson, unpublished data
[d] Mo *et al.* (37)
[e] Jung *et al.* (238)
[f] Elson and Mo (237)

phate pyrophosphatase activity (81, 209), and the cytosolic, mitochondrial and microsomal oxidase activities (87, 89, 92–96) that deplete cells of farnesyl pyrophosphate, one of the substrates for the transferase reaction (Fig. 1). A side note is our finding of a major water soluble, mevalonate-derived metabolite that is eluted immediately before farnesyl pyrophosphate from a reverse-phase ion pair HPLC column with a linear gradient (15%–60%) acetonitrile in 0.01 *M* tetrabutylammonium phosphate (158). Farnesyl pyrophosphate-derived α,ω-dibasic prenoic acids have similar solubility characteristics (94).

Mixed isoprenoids containing a farnesyl moiety e.g., γ- and δ- tocotrienol (53), menaquinone-3 (41), and farnesyl derivatives, e.g., farnesylthiosalicylic acid (233), farnesyl acetate (234), and farnesylamine (235) have greater *in vitro* tumor-suppressive potency than farnesol. The farnesyl moiety of the mixed isoprenoids and derivatives is not a substrate for the farnesol alcohol oxidoreductase activities (89). Therefore, these agents will likely have longer biological half-lives. Burke *et al.* (47) recently reported the first demonstration of an *in vivo* antitumor action of farnesol. A cross-study comparison of results published by Burke *et al.* (47) and He *et al.* (53) suggests that γ-tocotrienol has a substantially greater tumor-suppressive efficacy than farne-

Table II. IC_{50}[a] Values (μ*M*) for the Suppression of Human and Murine Tumor Cell Proliferation by an Array of Pure and Mixed (Tocotrienols) Isoprenoids

Isoprenoid	B16[a,b]	HL-60[a,c]	Caco-2[a,c]	MIA PaCa-2[d]	MCF-7[c,e]	HT-29[f]	MDA-MB-435[e]	A549[g]
d-Limonene	450 ± 43					5000		
Perillyl alcohol	250 ± 28			300		350		
Geraniol	150 ± 19			100				
Perillaldehyde	120 ± 17					50		
β-Ionone	140 ± 23	35 ± 6	55		60[a,c]			
Farnesol	50 ± 15			25				35
d-α-Tocotrienol	110 ± 15				15[e]		220	
d-γ-Tocotrienol	20 ± 3	4 ± 4			8[a,c]		75	
d-δ-Tocotrienol	10 ± 3				5[e]		220	
dl-α-Tocopherol	>1600				>300[e]		>440	

[a] The IC_{50} value is the concentration (±SD) of an isoprenoid required to suppress the net increase in tumor cell population by 50%. Isoprenoid effects were determined at midpoints on the linear growth curve determined for control cells.
[b] He *et al.* (53)
[c] Mo and Elson (40)
[d] Burke *et al.* (47)
[e] Guthrie *et al.* (221)
[f] Crowell *et al.* (156)
[g] Miquel *et al.* (45)

sol. The potential for applying γ-tocotrienol to the treatment of tumors is dimmed by findings that α-tocopherol markedly attenuates the impact of γ-tocotrienol. The two tocols likely compete for binding sites on plasma transport proteins (207).

Farnesol suppresses the proliferation of murine melanoma (53) cells with three-fold the potency of geraniol (Table II). Contrary to the wide differential recorded *in vitro*, dietary farnesol and geraniol equally suppressed the growth of implanted hamster pancreatic ductal adenocarcinomas (47). Geraniol also suppresses the growth of implanted P388 leukemia cells (51), Morris 7777 hepatomas (50), and B16 melanomas (50). Within-study comparisons of the *in vivo* actions of the two isoprenoids and their derivatives are not available. A cross-study comparison suggests that a geranylated coumarin, auraptene (236), has a substantially greater anticarcinogenic efficacy than geraniol (50). A review of IC_{50} values (Table I) reveals that the tumor growth–suppressive potency of geraniol is also markedly increased by blocking its oxidation (237). We have also evaluated the tumor growth–suppressive potencies of assorted derivatives of β-ionone (Table I). The most potent of the β-ionone derivatives listed in Table I, ethinyl and allyl carbinols, have three-fold the potency of the parent compound (238).

The growth curves providing the basis for calculating IC_{50} values are influenced by isoprenoid-mediated changes in rates of cell division (proliferation) and isoprenoid-induced cell death (apoptosis). Isoprenoids trigger time- and concentration-dependent actions at both levels (40). Isoprenoid-initiated apoptosis has been confirmed in B16, HL-60, and MCF-7 by TUNEL assay (40). Electrophoresis of DNA extracted from HL-60 cells incubated with the DNA topoisomerase 1 inhibitor camptothecin yields a laddering pattern consisting of segments differing by approximately 200 base pairs (197, 198). Electrophoretic patterns of DNA extracted from HL-60, Caco-2, and B16 cells harvested from isoprenoid-supplemented cultures revealed the generalized and diffuse segmenting DNA pattern (40) previously shown for DNA extracted from lovastatin-treated HL-60 cells (187). Apoptotic death is further characterized by blebbing of the cell surface as well as nuclear condensation and DNA breakage. Photomicrographs of asynchronous B16(F10) melanoma cells incubated for 24 hr (Fig. 2A) and 72 hr (Fig. 2B) show the contact-inhibition-free overgrowth of the control culture. The cells shown in Figure 2C were incubated in control medium for 24 hr (see Fig. 2A) and then with farnesyl tiglate for 48 hr (Fig. 2C). The concentration of farnesyl tiglate (100 μ*M*) applied to this culture is about double the agent's IC_{50} value, 58 ± 17 μ*M* (237). As a consequence, the cell population of the experimental culture after 48 hr incubation (Fig. 2C) is less dense than that of the 24-hr culture (Fig. 2A). The rounding of the cells and blebbing of the plasma membrane (Fig. 2C, arrows) precedes the release of apoptotic cells into the culture medium. These morphological changes are consistant with those noted in statin-treated fibroblasts (239). Previously noted were reports of isoprenoid-initiated tumor regression (38, 39). More recently the cyclic isoprenoid, perillyl alcohol, was shown to initiate apoptosis in liver tumors (240).

These isoprenoid-mediated changes in morphology are time- and concentration-dependent. The time-dependent onset of G1 arrest is tabulated in Table III. The results, we believe, show that the initial impact of the isoprenoid is the diversion of cells from G2/M toward apoptotic cell death. As progress through the G1 phase of the cell cycle wanes, the proportion of cells passing into G2/M decreases with a concomitant decrease in the proportion of cells being diverted toward apoptosis.

The results of a representative study of the concentration-dependent effects of β-ionone and lovastatin on the 24-hr cell cycle distribution of HL-60 cells (40) are listed in

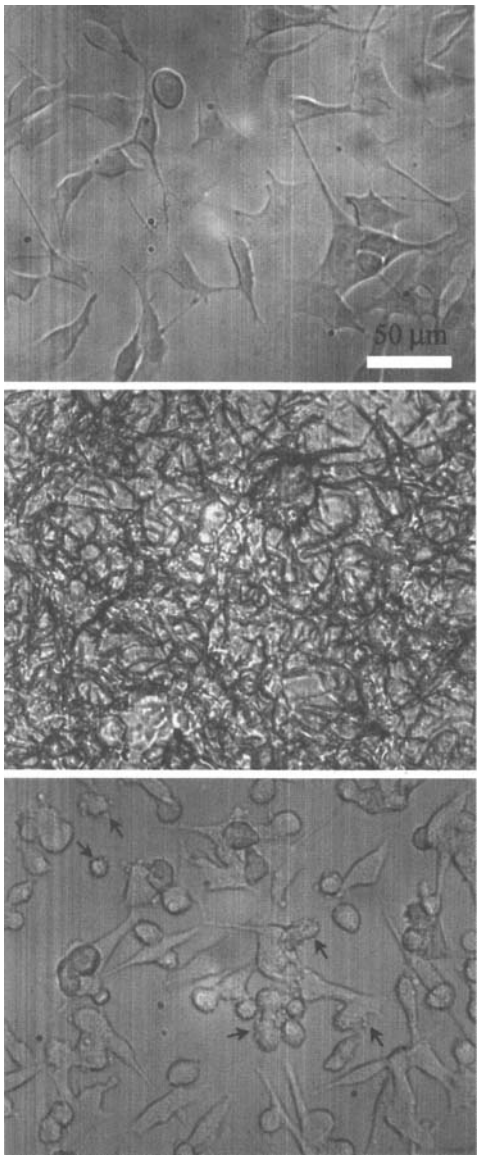

Figure 2. Photomicrographs of cultures of asynchronous B16(F10) melanoma cells incubated for 24 hr (Fig. 2A) and 72 hr (Fig. 2B) in control medium. The cells shown in Figure 2C were incubated in control medium for 24 hr (see Fig. 2A) and for an additional 48 hr in medium augmented with 100 μ*M* farnesyl tiglate. As apoptosis proceeds, cells round up, with nuclear fragmentation and plasma membrane blebbing (arrows) and detach from the monolayer. The medium containing the released cells was decanted prior to this analysis.

Table III. A Representative Analysis of the Time-Dependent Impact of β-Ionone (150 μ*M*) on Growth and Distribution of Murine B16(F10) Melanoma Cells in the Cell Cycle[a]

Time	% Apoptotic	Cell cycle distribution		
		G1	S	G2-M
0	2	66	30	3
1	3	67	25	7
2	4	67	25	8
3	7	67	27	5
8	5	78	15	7
12	3	88	10	2

[a] B16 cells were incubated with 150 μ*M* β-ionone for 0, 1, 2, 3, 8, and 12 hr. Cells remaining on the monolayer after washing were harvested and counted, and aliquots were analyzed by flow cytometry (40).

Table IV. Concentration-Dependent Impact of the Isoprenoid, β-Ionone, and the Competitive Inhibitor of HMG CoA Reductase, Lovastatin, on the 24-hr Distribution of HL-60 Cells in the Cell Cycle

β-Ionone (μ*M*)	% Apoptotic	Cell cycle distribution		
		G1	S	G2-M
0	0	40	50	10
20	0	40	50	10
50	12	48	50	2
75	75	84	11	5
Lovastatin (μM)				
0	0	40	50	10
0.05	0	43	46	11
0.10	66	45	45	10
0.20	70	71	23	3

[a] HL-60 cells were incubated with various concentrations of β-ionone (0-75 μ*M*) and lovastatin (0–0.2 μ*M*) for 24 hr. Cells were counted and cell cycle distribution analyzed by flow cytometry (40).

Table IV. Here, we show that the greater proportion of cells exposed to elevated levels of β-ionone for 24-hr is undergoing apoptotic cell death; those remaining viable are arrested in G_1.

Aberrent tumor growth can be traced to mutations of *ras* and p53, the former by sustaining cell proliferation and the latter, by blocking apoptosis. The isoprenoid-mediated suppression of cell growth is clearly independent of both mutated *ras* and p53 functions. β-Ionone suppresses the proliferation of tumor cells that either express wild-type (Caco-2 and MCF-7) or mutated (B16, HL-60) *ras*. Our finding that β-ionone also suppressed proliferation of cells that express wild-type *ras* confirms earlier work (181) showing that the tumor-suppressive action of cyclic isoprenoids, like that of lovastatin (180), is independent of *ras* function. Mevalonate synthesis is essential for the dolichol-supported glycosylation and membrane attachment of growth factor receptors (185, 186). Isoprenoids, we propose, suppress, with some selectivity, mevalonate synthesis, dolichol synthesis, and the glycosylation and membrane attachment of growth factor receptors; as a consequence, isoprenoid-treated cells may respond less vigorously to exogenous growth factors. The isoprenoid-mediated initiation of apoptosis is independent, also, of a mutated p53 function. Isoprenoids initiate apoptosis in cells that express wild-type (B16, MCF-7) and mutated (Caco-2) as well as in p53-null HL-60 cells (40).

The farnesyl pyrophosphate pool in cells incubated with lovastatin is limiting for the post-translational modification and function of neosynthesized lamins (145, 147, 149, 150, 195). Lovastatin has a dose-dependent impact on the growth of HL-60 cells (Table IV); the electrophoretic

pattern of DNA extracted from HL-60 cells following incubation with 1 μ*M* lovastatin reveals a generalized degradation (187) partially due to the presence of apoptosis-associated degraded lamin B (241), the predominant lamin expressed by HL-60 cells (149). The electrophoretic pattern of DNA extracted from β-ionone-treated (100 μ*M*) HL-60 cells revealed a similar pattern of generalized degradation: Western blots of lamin B immunoprecipitated from those cells revealed numerous lamin B fragments (40). The isoprenoid-mediated suppression of HMG CoA reductase activity leads to the suppressed processing of lamin B (40). We employed "no-effect" levels (Table IV) of β-ionone (20 μ*M*) and lovastatin (0.05 μ*M*) to evaluate their effects on lamin B processing independent of the confounding degradation of lamin B. Both agents suppressed (<50% of control) acetate-derived and enhanced (>175% of control) mevalonate-derived farnesylation of lamin B. The breakdown of lamin B processing subsequent to isoprenoid-mediated suppression of HMG CoA reductase activity, we postulate, blocks nuclear localization of lamin B, suppresses S phase DNA synthesis, and as a consequence of disrupting the reformation of daughter nuclei, renders neosynthesized DNA available to p53-independent apoptotic endonuclease activities.

It should be noted that proteins other than lamin B and *ras* respond to cyclic isoprenoids. Perillyl alcohol effectively increased the expression of pro-apoptotic BAK in pancreatic adenocarcinoma cells but not in nonmalignant pancreatic ductal cells (49). Further a subtractive display analysis of limone-treated mammary tumors detected 42 monoterpene-induced and 58 monoterpene-repressed genes (242). *In vitro* (231, 242, 243) and *in vivo* (244) studies record isoprenoid-induced differentiation. Other inhibitors of HMG CoA reductase activity similarly induce tumor cell differentiation (172). Although we perceive the isoprenoid-mediated alterations to be a consequence of cell cycle disruption subsequent to farnesol pyrophosphate starvation, we cannot rule out the possibility that isoprenoids initiate the aberrant expression of a gene that triggers cell cycle disruption and, concomitantly, suppresses HMG CoA reductase activity.

A secondary metabolite may be defined as a natural product that has no explicit role in the internal economy of the organism that produces it; yet it confer a survival advantage on the producing organism (245). The pure and mixed isoprenoids represent a subgroup of secondary products, examples of which are known to confer survival advantage to the producing organism (246–260). We return to the analogy that the sterol-feedback insensitive mevalonate pathway operative in fungi (118), yeast (119–125), protozoa (126), parasitic protozoa (126–128), helminths (126), and insects (129, 130), a putative target for the isoprenoid action, bears a striking similarity to the sterol-resistant mevalonate pathway activity in tumors.

Pure and mixed isoprenoids extracted from exotic botanicals, for example, taxol (261) and betulinic acid (262), have very potent antitumor activities. This review has emphasized the tumor-suppressive impact of pure and mixed isoprenoids widely present in fruits, vegetables, and cereal grains. Although it remains unlikely that an individual isoprenoid will be consumed in a quantity sufficient to modulate tumor growth, our studies provide evidence that the additive and/or synergistic impact of widely consumed isoprenoids may ultimately prove to be sufficient to account for the cancer risk–attenuating impact of a diet rich in plant products (1–10, 263).

This review presents a rationale for encouraging the consumption of fruits, vegetables, and cereal grains. The chemopreventive potential of monoterpenes has been emphasized in recent reviews (264–267). We elected to emphasize the tumor growth suppressive impacts of a carotenoid-derived pure cyclic isoprenoid and a mixed isoprenoid. The ionones and tocotrienols are widely present in plant-derived foods, intakes of which parallel dietary intakes of β-carotene and α-tocopherol. The ionones, principally β-ionone, are widely distributed in the fruits and vegetables, the consumption of which is associated with lower cancer risk. Fruit sources include blackberries (268), blueberries (269), cherries (268), citrus (270), grapes (271) and wine (272), peaches (273), raspberries (274–276), strawberries (277), and tomatoes (278–280). Vegetable sources include beans (281), broccoli (282), carrots (283), corn (284), and sweet potatoes (285). Among the other dietary constituents associated with lower cancer risk, tea (286, 287) and parsley (288), are sources of ionones. The tocotrienols, are generally present in high fiber cereals and grains [barley (28, 289), oats (289), rice (290), and wheat (291)] and oils extracted from olive (292–295) and palm fruit (296).

Guidelines promulgated for the purpose of reducing cancer risk (1–4) draw on epidemiologic data that associates cancer incidence with intake of fruits, vegetables, and cereal grains (6–9, 18, 297–299). In contrast to that strong association, there is little evidence to suggest that a supplementary intake of fiber (300–302), β-carotene (303–309), vitamin C (11, 303–305, 310–313), or vitamin E (11, 303–307, 310–313), reduces cancer risk. Supplementary folate, the remaining dietary component predominantly associated with fruit, vegetable, and cereal consumption, has in one (313) but not in a second (314) epidemilogic study shown to be related to reduced cancer incidence.

Rather than supporting the reductionist view that chemopreventive, or tumor-suppressive, relevancy is found in a singular action of a phytochemical or in a specific plant product, for example tomatoes or strawberries (315), our studies point to the additive and potentially synergistic tumor growth–suppressive actions of pure and mixed isoprenoids and other classes of the phytochemicals that are broadly distributed in plant products (34–37, 40).

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