MINIREVIEW

Studies of the Isoprenoid-Mediated Inhibition of Mevalonate Synthesis Applied to Cancer Chemotherapy and Chemoprevention

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Pools of farnesyl diphosphate and other phosphorylated products of the mevalonate pathway are essential to the post-translational processing and physiological function of small G proteins, nuclear lamins, and growth factor receptors. Inhibitors of enzyme activities providing those pools, namely, 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase and mevalonic acid–pyrophosphate decarboxylase, and of activities requiring substrates from the pools, the prenyl protein transferases, have potential for development as novel chemotherapeutic agents. Their potentials as suggested by the clinical responses recorded in Phase I and II investigations of inhibitors of HMG CoA reductase (the statins), of mevalonic acid–pyrophosphate decarboxylase (sodium phenylacetate and sodium phenylbutyrate), and of farnesyl protein transferase (R115777, SCH66336, BMS-214662, Tipifarnib, L-778,123, and, prematurely, perillyl alcohol) are dimmed by dose-limiting toxicities. These nondiscriminant growth-suppressive agents induce G1 arrest and initiate apoptosis and differentiation, effects attributed to modulation of cell signaling pathways either by modulating gene expression, suppressing the post-translational processing of signaling proteins and growth factor receptors, or altering diacylglycerol signaling. Diverse isoprenoids and the HMG CoA reductase inhibitor, lovastatin, modulate cell growth, induce cell cycle arrest, initiate apoptosis, and suppress cellular signaling activities. Perillyl alcohol, the isoprenoid of greatest clinical interest, initially was considered to inhibit farnesyl protein transferase; follow-up studies revealed that perillyl alcohol suppresses the synthesis of small G proteins and HMG CoA reductase. In sterologenic tissues, sterol feedback control, mediated by sterol regulatory element binding proteins (*SREBPs*) *1a* and *2*, exerts the primary regulation on HMG CoA reductase activity at the transcriptional level. Secondary regulation, a nonsterol isoprenoid-mediated fine-tuning of reductase activity, occurs at the levels of reductase translation and degradation. HMG CoA reductase activity in tumors is elevated and resistant to sterol feedback regulation, possibly as a consequence of aberrant *SREBP* activities. Nonetheless, tumor reductase remains sensitive to isoprenoid-mediated post-transcriptional downregulation. Farnesol, an acyclic sesquiterpene, and farnesyl homologs, γ-tocotrienol and various farnesyl derivatives, inhibit reductase synthesis and accelerate reductase degradation. Cyclic monoterpenes, *d*-limonene, menthol and perillyl alcohol and β-ionone, a carotenoid fragment, lower reductase mass; perillyl alcohol and *d*-limonene lower reductase mass by modulating translational efficiency. The elevated reductase expression and greater demand for nonsterol products to maintain growth amplify the susceptibility of tumor reductase to isoprenoids, therein rendering tumor cells more responsive than normal cells to isoprenoid-mediated growth suppression. Blends of lovastatin, a potent nondiscriminant inhibitor of HMG CoA reductase, and γ-tocotrienol, a potent isoprenoid shown to post-transcriptionally attenuate reductase activity with specificity for tumors, synergistically affect the growth of human DU145 and LNCaP prostate carcinoma cells and pending extensive preclinical evaluation, potentially offer a novel chemotherapeutic strategy free of the dose-limiting toxicity associated with high-dose lovastatin and other nondiscriminant mevalonate pathway inhibitors. Exp Biol Med 229:567–585, 2004

Key words: isoprenoid; 3-hydroxy-3-methylglutaryl coenzyme A reductase; γ-tocotrienol; lovastatin; perillyl alcohol; cell cycle arrest; apoptosis

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The mevalonate pathway, also known as the cholesterol pathway, produces cholesterol and a number of nonsterol products. While cholesterol has been extensively studied for its implication in cardiovascular disease, findings that nonsterol products of the mevalonate pathway are essential for cell survival suggest that the pathway also offers novel targets for cancer chemoprevention and chemotherapy (1). Figure 1 provides an orientation to the mevalonate pathway with reference to activities, 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase, mevalonic acid–pyrophosphate decarboxylase, and farnesyl protein transferase, considered to have potential for chemotherapeutic intervention. Although agents targeted to these activities have proved to be effective in animal studies, their promise in clinical trials is clouded by dose-limiting toxicities. HMG CoA reductase, the rate-limiting activity in the mevalonate pathway, is downregulated by post-transcriptional actions triggered by mevalonate-derived end products of plant mevalonate metabolism (isoprenoids; Ref. 1). An anomaly associated with tumor growth—a sterol feedback resistant and overexpressed HMG CoA reductase activity—renders the mevalonate pathway in tumors uniquely sensitive to isoprenoid-mediated post-transcriptional downregulation (1).

First reviewed are results of Phase I and II clinical trials of agents targeted to sites identified on Figure 1. Next, the parallel and conflicting actions imposed by selected isoprenoids and by lovastatin, the most prominently studied of the statins, that potentially affect tumor growth are briefly noted. Following a brief update of the progress in delineating of the error underlying the dysregulation of HMG CoA reductase activity in tumor tissues, isoprenoid-mediated actions underlying the suppression of reductase activity with specificity for tumor cells are reviewed. The resulting depletion of nonsterol products of the mevalonate pathway, we suggest, is responsible for many of the isoprenoid-mediated actions listed in Table 1.

Pools of mevalonate-derived products, farnesyl diphosphate, geranylgeranyl diphosphate, and dolichyl phosphate, are essential for the post-translational modification and biological activity of diverse proteins that have roles in cell growth (Fig. 1). As a consequence, mevalonate pathway activities are potential targets for novel chemotherapeutic agents (1). One approach employs the competitive inhibition of HMG CoA reductase activity imposed by a statin (fluvastatin, atorvastatin, cerivastatin, rosuvastatin, pitsvastatin, pravastatin, simvastatin, or lovastatin; Fig. 1). Lovastatin impedes cell proliferation (2). This effect was initially attributed to a rate-limiting pool of cholesterol with a concomitant attenuation of membrane assembly. The seminal finding of the incorporation of mevalonate-derived products into animal proteins (3) suggested an alternative rationale for explaining the impact of statins on cell proliferation. Investigators first identified the carboxyterminal cysteine residue of lamin B (4) and immediately thereafter that of p21*ras* as the site where a mevalonate-derived farnesyl group is covalently attached (5). This processing is essential for the biological function of the nuclear lamins (6) and virtually all members of the Ras superfamily of proteins (5). As a consequence of mevalonate starvation, cells incubated with statins accumulate in the G1 phase of the cell cycle or undergo apoptotic death (7–12).

The early observations of low concentrations of cholesterol and ubiquinone-10 in Phenylketonuria (PKU) patients led to the finding that phenylacetate inhibits mevalonic acid–pyrophosphate decarboxylase activity (Fig. 1), a key activity in the synthesis of the aforementioned prenyl diphosphates (13). As a consequence, phenylacetate suppresses the incorporation of radiolabeled mevalonate into cellular proteins (14). Sodium phenylacetate and sodium phenylbutyrate arrest cell growth with cells accumulating in the G1 phase of the cell cycle (14–16).

Inhibition of HMG CoA reductase or mevalonic acid–pyrophosphate decarboxylase suppresses the syntheses of farnesyl diphosphate and geranylgeranyl diphosphate, the substrates providing the isoprenoid moieties required for the post-translational modification of the cysteine residue in the conserved carboxyl terminus sequence (generally CAAX) of diverse proteins, some of which have roles in signal transduction (Ras, Rho) and cytoskeletal organization. More recent interest has focused on the inhibition of farnesyl protein transferase, the activity catalyzing the transfer of the farnesyl moiety to the aforementioned proteins (Fig. 1). A recent review of the effects of various farnesyl protein transferase inhibitors cited changes in the cell cycle with cells accumulating in G1 in some cell lines and G2 in others and the induction of apoptosis (17). Therapeutic strategies targeted to the three activities delineated above (see Fig. 1) are in clinical evaluation.

Clinical Studies

Inhibition of HMG CoA Reductase: Lovastatin. Although preclinical evaluations demonstrated the significant chemotherapeutic potential of high-dose statins (15–70 mg/kg body weight), the results of clinical trials have shown only modest promise. Significant clinical responses (stable or regressing anaplastic astrocytoma, glioblastoma multiforme, gastric adenocarcinoma) were reported for 5 of 35 patients enrolled in Phase I (18), Phase I–II (19), and Phase II (20) trials. Briefly, the patients tolerated lovastatin at doses to 25 mg/(kg·day) (20); clinical responses required doses of 20–35 mg/(kg·day). In each of the preceding studies, lovastatin was dosed for 1 week with a 3-week follow-up period. Drug-related toxicities halted a study wherein the protocol called for 10–20 mg/(kg·day) lovastatin to be administered to acute myelogenous leukemia patients for 2 weeks (21). Toxicities associated with high-dose lovastatin include gastrointestinal dysfunction, myalgia, muscle weakness, elevated creatine phospho-

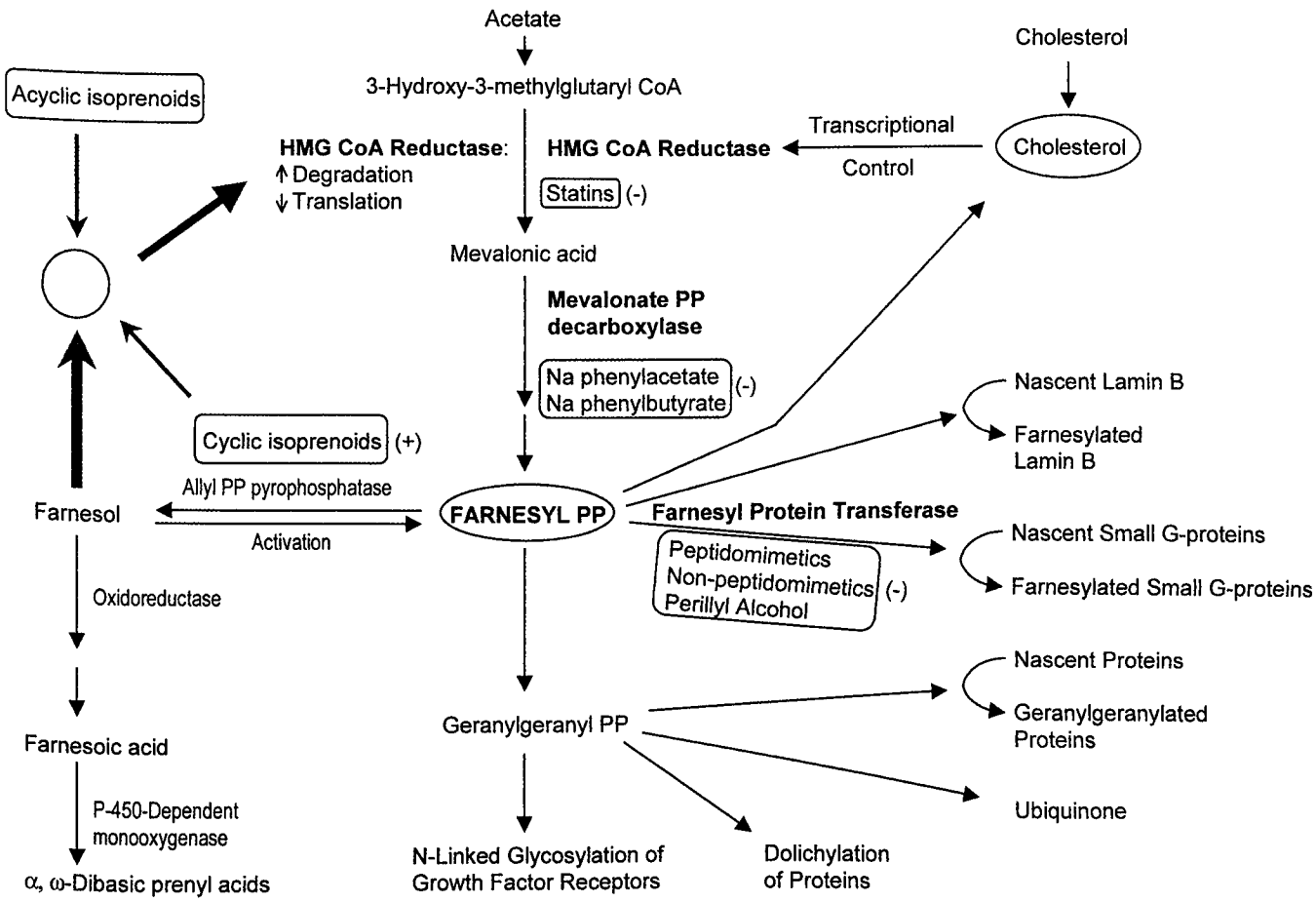

Figure 1. Overview of the mevalonate pathway with reference to the nonsterol products required for the post-translational processing and biological activity of proteins involved in growth factor signaling and enzyme activities targeted for pharmacological intervention. Also shown is an overview of transcriptional, translational, and post-translational actions initiated by mevalonate-derived products of endogenous (cholesterol, farnesol) and exogenous (acyclic and cyclic isoprenoids) origin that modulate HMG CoA reductase activity. Figure 2 shows the differential regulation of reductase activity in sterologenic and tumor cells.

kinase, anorexia, and ulcerative lesions. Doses approaching levels normally prescribed for cholesterol control (20–80 mg/day) may have efficacy. A 5-year follow-up study of the safety of lovastatin recorded a 33% lower than predicted incidence of cancer (22). A recent nested control study comparing cancer incidence in a hypercholesterolemic population treated with bile-acid binding resins with that of a population treated with a statin (lovastatin, pravastatin, simvastatin) recorded a 28% lower diagnosis of any cancer among patients in the latter group (23). Pravastatin (40 mg/day) doubled the median survival time for elderly hepatocellular carcinoma patients. Side effects were limited to liver dysfunction (24). The rarely reported peripheral neuropathies associated with low-dose statin treatments are readily reversed following discontinuation of the drugs (25, 26). A recent summary of the results of clinical trials investigating the potential value of statins alone and in combination with other agents as chemotherapeutic agents concludes that further testing is merited (27).

Inhibition of Mevalonic Acid–Pyrophosphate Decarboxylase: Sodium Phenylacetate, Sodium Phenylbutyrate. Although preclinical evaluations demonstrated the significant chemotherapeutic potential of high-dose sodium phenylacetate (550 mg/kg body weight; Refs. 13, 14), the results of clinical trials have shown less promise. Sodium phenylacetate and sodium phenylbutyrate, inhibitors of mevalonic acid–pyrophosphate decarboxylase activity, have been evaluated in Phase I (28–31) and Phase II (32, 33) studies. Although modest responses were recorded with circa 300–400 mg/kg/day infusions (30–33), dose-limiting toxicities including reversible central nervous system depression, preceded by emesis (30, 31), reversible Grade 2 or 3 neurocortical toxicity, consisting of transient somnolence, confusion, exacerbation of an underlying seizure disorder (32), fatigue, and somnolence were the primary toxicities, with only mild hematologic toxicity (33).

Inhibition of Farnesyl Protein Transferase: R115777, SCH66336, BMS-214662, Tipifarnib, and L-778,123. Four nonpeptidomimetic farnesyl protein transferase inhibitors, R115777 (34–39), SCH66336 (40–43), BMS-214662 (44), and tipifarnib (45), are undergoing clinical trials with each achieving significant clinically

Table 1. Reports of the Modulation of Mechanisms Potentially Underlying the Tumor-Suppressive Actions of Cyclic and Acyclic Isoprenoids, Acyclic Isoprenoid Derivatives, and Lovastatin[a]

	Isoprenoids			
Response recorded	Cyclic	Acyclic	Acyclic derivatives	Lovastatin
Suppresses cell growth	65, 67, 74	69	68, 102	193
Suppresses cell growth with specificity for tumors	65, 67, 74	69	68, 102	
Initiates G1 arrest	83–86	79–82	68, 78, 194	68, 132, 195
Suppresses N-linked glycosylation	196	196		196–199
Induces $p21^{Cip1/WAF1}$ expression	66, 86, 126			12, 122, 132, 133, 136, 138
Suppresses cyclin D1 expression	83, 86, 126			122, 129, 133, 134
Suppresses Cdk-2 expression/activity	66, 126, 133			138, 200
Suppresses Cdk-2 phosphorylation	86			133, 141
Suppresses cyclin D/Cdk activity	86, 126			122, 140
Suppresses pRb phosphorylation	86			12, 132, 133, 136, 140
Suppresses cyclin E expression	66			130, 131, 133, 134
Induces cyclin E expression	126			
Suppresses c-*myc* expression	128			129
Induces c-*fos* expression	66, 127			137
Suppresses Cdk-4 expression	126			122, 200
Suppresses E2F activity			73	129
Suppresses cyclin E/Cdk-2 activity			201	136
Induces $p27^{Kip1}$ expression			194, 201	122, 130, 131, 134, 136, 138
Initiates apoptosis	66, 84, 94–96, 202	65, 69, 70, 87–90	68, 73, 75, 79, 91–93, 194, 203	68, 195, 204–208
Suppresses Bcl-2 expression	124	125		108, 130, 131, 204, 207
Suppresses Bcl-xL expression	125	125		135
Induces *bax* expression	66			207
Induces *bad* expression	66			
Induces annexin I expression	66			
Induces TGFβ-related genes	66			
Induces manose-6-phosphate/IGF-II receptor expression	66, 94, 95, 146			
Induces caspase-3 activity		209	194, 210, 211	
Induces caspase-7 activity				212
Induces caspase-8 activity			211	
Induces differentiation		213, 214	194	204, 208
Impacts HMG CoA reductase activity				
Suppresses HMG CoA reductase activity	145, 168	49, 145, 161–163	165, 215, 216	155
Blocks statin-mediated upregulation of HMG CoA reductase	145, 217	49, 161, 163	165	
Suppresses HMG CoA reductase synthesis	145	145	216	
Reduces HMG CoA reductase mRNA level		145		
Suppresses HMG CoA reductase activity with specificity for tumors			165	
Induces HMG CoA reductase expression				155
Impacts small G proteins				
Suppresses small G protein isoprenylation	49, 51, 74, 107, 112–117		73, 99, 100	108–110, 123, 218
Suppresses statin-mediated upregulation of Ras, small G proteins	111, 116, 190			

Table 1. Continued

Response recorded	Isoprenoids			
	Cyclic	Acyclic	Acyclic derivatives	Lovastatin
Suppresses small G protein synthesis	218			
Induces RhoA, RhoB expression			189	111, 122, 123, 189
Induces Ras expression			110, 189	111, 122, 123, 189
Suppresses prenyl transferase activities				
Suppresses farnesyl protein transferase activity			219	
Suppresses geranylgeranyl protein transferase activity	113			
Suppresses farnesylation of OV-1 protein tyrosine phosphatase	114			
Impacts signal transduction				
Suppresses MAPK/ERK activity	74		71, 121	139
Induces BAK expression	65, 125	125		
Suppresses protein kinase C activity		72, 87, 151		
Increases c-*jun* expression and activity	127		73	
Suppresses PI3-kinase/Akt activity			73	220
Increases c-*jun* phosphorylation	127			
Induces AP-1 expression	127			
Suppresses AP-1 binding				109, 221
Suppresses PI3 production				222
Suppresses *raf*-1 activity				222
Others				
Acidifies cytoplasm	202			
Redistributes phosphatidylserine	202			
Suppresses ornithine decarboxylase activity		223		
Suppresses choline phosphotransferase activity		90, 151		
Induces polyamine acetylation		223		
Suppresses CTP:phosphocholine cytidyltransferase α		154		
Suppresses prenylated protein methyl transferase activity			71	

[a] All numbers in the table are references.

relevant responses; severe gastrointestinal and liver toxicities prevented the achievement of adequate systemic exposures following oral exposure to BMS-214662 (46). Shared dose-limiting toxicities include nausea, vomiting, and diarrhea. Agent-specific toxicities include myelosuppression, neurological complications, and skin sensitivity (R115777); myelosuppression and renal and neurological complications (SCH66336); abdominal cramping, anorexia, fatigue, and fever (BMS-214662); and myelosuppression (tipifarnib). Clinical investigations of a peptidomimetic farnesyl protein transferase (FT) inhibitor (L-778,123) have been discontinued because of evidence of cardiac conduction abnormalities (44, 47, 48).

Inhibition of Farnesyl Protein Transferase: Perillyl Alcohol. Diverse mevalonate-derived products of secondary metabolic pathways present in plants (pure and mixed isoprenoids) have both chemotherapeutic and chemopreventive properties (1). Early studies of the mechanism of the anticarcinogenic actions of two pure isoprenoids, *d*-limonene and perillyl alcohol, revealed that these cyclic monoterpenes suppressed the incorporation of radiolabeled mevalonate into small G proteins (49–51). This action was widely attributed to the inhibition of farnesyl protein transferase activity. Phase I studies of *d*-limonene (52, 53) and Phase I (54–58) and II (59–61) studies of perillyl alcohol revealed dose-limiting toxicities; nausea, vomiting, anorexia, unpleasant taste, and eructation. Hypokalemia due to decreased absorption of dietary potassium was reversed with modest oral potassium supplementation (55). The maximum tolerated dose determined for perillyl alcohol was determined to be 8.4 g/(M^2·day) delivered orally in four doses (54). Modest clinical responses were attained in trials with doses ranging from 2.1 g/M^2 delivered in 3 doses (56), 2.4 g/M^2 delivered in 3 doses (57), and 4.8 g/M^2 delivered in

Table 2. Reports of Shared Impacts on Cell Proliferation and Activities Associated with G1 Arrest and Initiation of Apoptosis Imposed by Perillyl Alcohol and Lovastatin[a]

Response recorded	Perillyl alcohol	Lovastatin
Suppresses cell growth	65, 67, 74	193
Initiates G1 arrest	83–86	68, 132, 195
Initiate apoptosis	66, 84, 94–96, 202	68, 195, 204–208
$p21^{Cip1/WAF1}$ induction	66, 86, 126	12, 122, 132, 133, 136, 138
c-*fos* induction	66, 127	137
Cyclin D1 suppression	83, 86, 126	122, 129, 133, 134
Cdk-2 suppression	66, 126, 133	138, 200
Cdk-4 suppression	126	122, 200
Cyclin E suppression	66	130, 131, 133, 134
c-*myc* suppression	128	129
Bcl-2, Bcl-xL suppression	124, 125	108, 130, 131, 135, 204, 207
Induces *bax* expression	66	207
Suppresses pRb phosphorylation	86	12, 132, 133, 136, 140
Suppresses Cdk phosphorylation	86	133, 141
Suppresses Cyclin D/Cdk activity	86, 126	122, 140
Suppresses small G protein isoprenylation	49, 51, 74, 107, 112–117	108–110, 123, 218
Suppresses MAPK/ERK activity	74	139
Suppresses N-linked glycosylation	196	196–199

[a] All numbers in the table are references.

4 doses (58) but not in all trials (59–61). Efforts are now directed toward building high-dose formulations of perillyl alcohol free of gastrointestinal toxicity (62). In a recent dose escalation study, patients received 2 to 5 capsules, each containing 675 mg perillyl alcohol, 4 times per day over a 28-day cycle. Gastrointestinal discomfort associated with the maximum dose, about 9 g/M^2, was not dose limiting (62).

Experimental Studies

Isoprenoids differ substantially in tumor-suppressive potency, and diverse tumor cell lines differ substantially in sensitivity to an individual isoprenoid (63, 64). Although the concentration-dependent impact of pure, mixed, and derivatized isoprenoids on the growth of cultured cells, with selectivity for tumor cells (65–77), has been broadly demonstrated, there remains uncertainty regarding the initiating event triggered by isoprenoids (78). Tumor growth, for this discussion, reflects only an increase in cell population. Growth reflects a "positive" balance between two factors, cell division and cell death, that occurs when the rate of division exceeds the rate of death. More definitive studies employing cell cycle analysis demonstrate that isoprenoids affect both sides of the "balance" equation (78). Farnesol (79–81), geraniol (82), β-ionone (68), perillyl alcohol (83–86), perillaldehyde (84), and γ-tocotrienol (68) slow the progress of diverse lines of tumor cells through the cell cycle with a resultant buildup of cells in the G1 phase. On the other side of the equation, farnesol (65, 69, 70, 87–90), farnesyl derivatives (73, 91–93), geraniol (82), β-ionone (68), limonene (94), perillyl alcohol (66, 84, 95, 96), and γ-tocotrienol (68) initiate apoptotic cell death.

Investigations of the mechanisms underlying the tumor growth-suppressive actions of isoprenoids, G1 arrest, apoptosis, and differentiation have largely focused on the modulation of cell signaling pathways either by altering gene expression, post-translational processing (phosphorylation, farnesylation, geranylgeranylation) of expressed proteins, or diacylglycerol signaling. Listed in Table 1 are reports of cellular responses to isoprenoid end products of plant mevalonate metabolism, the widely studied cyclic monoterpenes, *d*-limonene and perillyl alcohol, the less widely studied acyclic monoterpenes (geraniol) and sesquiterpenes (farnesol), and farnesyl derivatives. Also listed are reports of cell growth-suppressive actions of lovastatin affecting G1 arrest, apoptosis, and differentiation. We identify reports of 27 activities that respond similarly to isoprenoids and lovastatin (Table 1). Reports showing parallel impacts of perillyl alcohol, the isoprenoid of greatest clinical interest, and lovastatin on HMG CoA reductase activity, cell proliferation, and 15 activities associated with G1 arrest and initiation of apoptosis are listed in Table 2.

Synthetic (farnesylamine, Refs. 73, 97, 98; farnesylthiosalicyclic acid, Ref. 99; farnesylpyridinium, Ref. 100; farnesyl anthranilate, Ref. 92; farnesyl-*O*-acetylhydroquinone, Ref. 93; farnesyl thioacetate, Ref. 91) and natural (tocotrienols, Refs. 68, 101, 102; menaquinoine-3, Refs. 103, 104) agents containing a farnesol moiety suppress tumor cell growth with greater potency than farnesol, a difference we attribute to the masking of the farnesol head group from the cytosolic and microsomal activities that convert farnesol to dibasic prenoic acids (1). Alternatively, masking the head group prevents the reactivation of farnesol, that is, its conversion to farnesyl diphosphate (105, 106).

Suppression of Protein Isoprenylation

The wide variety of responses listed in Table 1 may be a result of the suppression of the post-translational modification of signal transduction proteins, Ras and Rho, either by the competitive inhibition of protein prenyl transferase activities or by depletion of the prenyl diphosphate pools; as a consequence the nascent Ras and Rho lack oncogenic activity.

Initial findings that perillyl alcohol suppressed the incorporation of radiolabeled mevalonate into small G proteins in mevalonate-deprived cells, for example, in cells incubated with lovastatin, suggested that the monoterpene inhibited farnesyl protein transferase activity (51). Follow-up studies comparing the potencies of perillyl alcohol metabolites with the parent compound determined that the monoterpenes did not directly inhibit the enzyme, farnesyl protein transferase (107). Depletion of the farnesyl diphosphate pool secondary to lovastatin-mediated mevalonate depletion attenuates the farnesylation of Ras (108, 109) and signals the upregulation of the small G proteins (110, 111). Perillyl alcohol decreases the lovastatin-mediated upregulation of the small G proteins (110, 111). As a consequence in lovastatin-treated cells incubated with perillyl alcohol there is a smaller pool of small G proteins available for labeling, which likely accounts for reports of the perillyl alcohol–mediated suppression of the isoprenylation of small G proteins (49, 51, 74, 112–117).

The potency of diverse monoterpenes in suppressing protein isoprenylation parallels their potency in suppressing tumor cell proliferation (49), although the latter action requires substantially lower concentrations of the metabolites. Monoterpenes suppress the prenylation of other proteins that may be oncogenic, for example, Ras-related TC21/R-Ras2 (118) and the PRL-1/PTP(CAAX) tyrosine phosphatases (114). In one study, perillyl alcohol arrested the growth and initiated apoptosis in Bcr/Abl-transformed hematopoietic cells in the absence of an inhibitory impact on Ras prenylation and Ras activity (74).

Farnesylamine (73), but not the sesquiterpene, *trans-trans*-farnesol (90) affects the prenylation of small G proteins. Cellular kinases may provide substrate for the farnesylation of the small G proteins by activating farnesol (105, 106). Another derivatized form of farnesol, farnesylthiosalicyclic acid, is variously reported to inhibit, *in vitro*, methyltransferase activity, the final step in the post-translational processing of Ras (99), to prevent the membrane attachment of mature Ras (119), and to dislodge membrane-bound Ras (120, 121), therein facilitating its degradation. Differing from farnesol, another derivative, farnesylpyridinium, appears to have detergent-like properties (100).

Modulation of Gene Expression

Changes in cellular levels of small G proteins mediated by lovastatin, perillyl alcohol, and geraniol do not reflect the inhibitory impact of these agents on tumor cell proliferation. Lovastatin upregulates the expression and cellular levels of Ras and Ras-related proteins (RhoA, RhoB, Rap1a; Refs. 110, 111, 122, 123). Perillyl alcohol lowers cellular levels of Ras, Rap1a, RhoA, and RhoB levels and attenuates the lovastatin-mediated upregulation of Ras and Ras-related proteins (111). Whereas geraniol is as potent as perillyl alcohol in attenuating the growth of leukemia cells, the acyclic monoterpene has no impact on Ras, Rap1a, RhoA, and RhoB levels (111). A study examining the effects of diverse monoterpenes on Ras, Rap1a, RhoA, and RhoB protein levels in the presence and absence of mevalonate depletion found no relationship between monoterpene-mediated impacts on small G protein levels and cell proliferation (111).

Contrary to their opposite impacts on cellular Ras, Rap1a, RhoA, and RhoB levels for both perillyl alcohol (66, 83, 86, 94, 124–128) and lovastatin (12, 108, 110, 122, 123, 129–138) suppress the expression of antiapoptotic activities and activities required for cell cycle progression (cyclin E, Refs. 66, 130, 131, 133, 134; cyclin-dependent kinase-2 [Cdk-2; Refs. 66, 126, 130, 138]; Bcl-xL, Refs. 125, 135; cyclin D1, Refs. 83, 86, 122, 126, 133, 134; c-*myc*, Refs. 128, 129) and induce expression of proapoptotic activities, $p21^{Cip1/WAF1}$ (12, 66, 86, 126, 132, 133, 136, 138), and transforming growth factor-β (TGF-β; Table 2; Refs. 12, 66, 126). Perillyl alcohol (74, 86, 126) and lovastatin (12, 73, 132, 133, 136, 139–141) are reported to suppress phosphorylation (activation) of Cdk (86, 133, 141), phosphorylation (activation) of the retinoblastoma tumor-suppressor protein pRB (12, 86, 132, 133, 136, 140), cyclin D1 Cdk-2 binding (86, 126, 140), and Mek/Erk activity (73, 74, 139).

Since the isoprenoid-mediated suppression of tumor cell proliferation does not require the presence of *ras* oncogenes (68), other cell cycle–relevant proteins that require post-translational modification may be relevant. Progression of cells through G1 into S phase requires the activation of Cdk activities, activities inhibited by Cdk-inhibitory proteins. Inhibition of RhoA prenylation by a geranylgeranyl transferase inhibitor (GGTi-298) results in an accumulation of one Cdk-inhibitory protein, $p21^{Cip1/WAF1}$,in tumor cells (142, 143). Cellular levels of $p21^{Cip1/WAF1}$ and a second Cdk inhibitor, $p27^{Kip1}$, are increased by lovastatin (12, 144). These findings suggest that lovastatin-mediated mevalonate depletion sequentially blocks RhoA prenylation, increases cellular cdk-inhibitor levels, and blocks the activation of cyclin E/Cdk-2 and cyclin D/Cdk-4, activities that are essential for cell cycle progression and cellular proliferation.

Cyclic monoterpenes also block RhoA prenylation, either by inhibiting geranylgeranyl transferase (113) or by suppressing mevalonate synthesis (145). The resulting increase in $p21^{Cip1/WAF1}$ (66, 86, 126) blocks the activation of cyclin E/Cdk-2 and cyclin D/Cdk-4, causing the arrest of cells in G1 (Table 1).

Antiapoptotic cyclin E, Cdk-2 (66), and cyclin D1 (83, 86, 126) activities are repressed, and proapoptotic activities,

$p21^{Cip1/WAF1}$ (66, 86, 126), *bad* (66), *bax* (66), Bak (65, 125), Annexin I (66), mannose-6-phosphate (M-6-P)/insulin-like growth factor II (IGF-II) receptor (66, 94, 95, 146), and TGF-β1 (66) are increased in tumors and tumor cells exposed to perillyl alcohol (Table 1). Nascent TGF-β1, secreted from cells as a complex containing phosphomannosyl residues, binds to M-6-P/IGF-II receptors where proteolytic activation occurs. The M-6-P/IGF-II receptor therefore functions both by activating a mitoinhibitor (TGF-β1) and by degrading a potent mitogen (IGF-II). The M-6-P/IGF-II receptor gene has been identified as a tumor-suppressor gene in liver and mammary tumors. Mammary tumors that regressed following exposure of the hosts to a diet containing 10% *d*-limonene had increased levels of both M-6-P/IGF-II receptors and TGF-β1; the increase in M-6-P/IGF-II receptor appeared to result from alterations at both transcriptional and post-transcriptional levels (94). Subsequent studies confirmed the monoterpene-induced increase in M-6-P/IGF-II receptor mRNA in regressing mammary (66, 146) and liver (95) tumors. TGF-β Type 1 and 2 receptors mRNAs in liver tumors (95) and mammary carcinomas (66) responding to perillyl alcohol were significantly increased when compared to levels in surrounding tissues. TGF-β autoinduction is mediated by the activator protein-1 (AP-1) complex (147); perillyl alcohol transiently induced the expression of growth-associated genes, c-*jun* and c-*fos*, components of AP-1. The impact of perillyl alcohol on c-*fos* and c-*jun* expression and c-*jun* phosphorylation was dose dependent with minimal impacts noted with 0.5 m*M* perillyl alcohol and maximum impacts attained with 2.0 m*M* perillyl alcohol. AP-1–dependent transcription, however, occurred maximally with 0.5 m*M* perillyl alcohol (127). Dietary *d*-limonene blocked the overexpression of c-*jun* at both mRNA and oncoprotein levels in livers of mice following *N*-nitrosdiethylamine administration (128). TGF-β initiates cytosis (148) through the inhibition of cyclin-Cdk complex activities (149), which is partly due to the induction of Cdk inhibitor $p21^{Cip1/WAF1}$ (86, 150), which binds and regulates the cyclin E-Cdk-2 complexes; blocking the formation of the cyclin D1-Cdk complex blocks pRB phosphorylation (86).

Modulation of Diacylglycerol Signaling

The farnesol-mediated inhibition of cell growth (69) is reversed with diacylglycerol, a phosphatidylcholine-derived metabolite and a second messenger in protein kinase C–dependent signal transduction (69, 151). Further, cells cultured in choline-deficient medium accumulate in G1 (152). These findings led to the demonstration that farnesol competitively inhibits choline phosphotransferase, the final activity in phosphatidylcholine biosynthesis (90). Another study finds the competitive inhibition of choline phosphotransferase likely to be the initiating event in the farnesol-mediated suppression of cell proliferation (90, 153); the IC_{50} (concentration required to suppress growth by 50%) value for both actions is about 40 μ*M* (90). However, the overexpression of choline phosphotransferase overcame the farnesol-mediated inhibition of phosphatidylcholine synthesis but did not prevent farnesol-induced apoptosis (151). More recently, farnesol-induced apoptosis was shown to cause a dose-dependent activation of CTP:phosphocholine cytidylyltransferase α (CCT α), the key step in the pathway, resulting in a biphasic effect on phosphatidylcholine synthesis. Activation of CCT α was accompanied by enzyme translocation to the nuclear envelope within 30 mins of farnesol addition to cells. Following translocation to membranes, CCT α was exported from the nucleus and underwent caspase-mediated proteolysis that coincided with poly (ADP-ribose) polymerase cleavage (154). An alternative hypothesis consistent with these findings is that diacylglycerol-regulated protein kinase Cα and Cβ isoforms are translocated from their active membrane-bound forms to cytosolic storage forms upon exposure to farnesol (72, 87).

Isoprenoid-Mediated Inhibition of Mevalonate Synthesis

Inhibition of the mevalonate pathway by lovastatin, a competitive inhibitor of HMG CoA reductase activity (155), profoundly alters the proliferative and differentiation characteristics of a wide variety of normal and malignant mammalian cell types. For many of the intracellular proteins that regulate proliferation and differentiation, covalent linkage to either farnesyl diphosphate or geranylgeranyl diphosphate is essential for normal regulating activities. As a consequence of the lovastatin-imposed depletion of farnesyl and geranylgeranyl diphosphate pools, the essential post-translational processing of intracellular proteins that regulate proliferation and differentiation is suppressed. Cyclic monoterpenes, acyclic monoterpenes and sesquiterpenes, and natural and synthetic farnesylated analogues also affect activities that modulate cell proliferation and differentiation (Table 1). The geraniol-mediated arrest of tumor cell proliferation (82), like that imposed by lovastatin (12, 129), is reversed with supplemental mevalonate.

In normal cells, HMG CoA reductase activity, the direct target of lovastatin and the rate-limiting activity of the mevalonate pathway, is subject to complex feedback regulation at the transcriptional, translational, and post-translational levels by both the sterol end product, cholesterol, and by a nonsterol product derived from the mevalonate pathway (Fig. 2A; Ref. 155). Sterol-mediated regulation of transcription, the dominant regulatory site in sterologenic tissues, is mediated through the sterol regulatory element (SRE), a promoter-enhancer sequence located in the 5′ flanking region of the reductase gene. The binding of mature SRE binding proteins (*SREBP-1a, SREBP-2*) interacting with transcriptional coactivators CBP and P300 within the SRE region is required for activation of HMG CoA reductase transcription (156). High-level

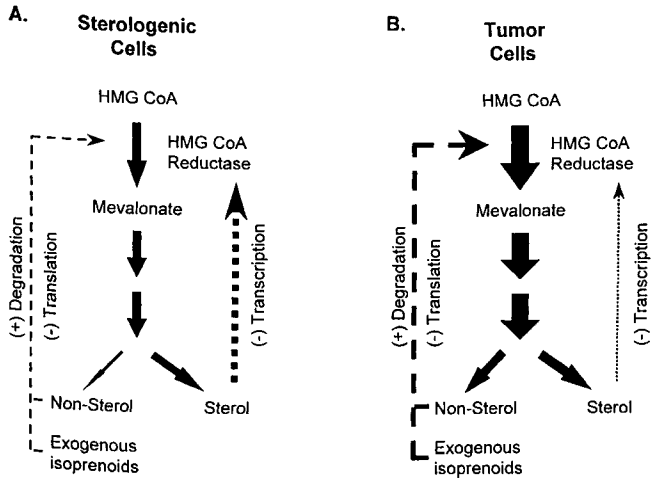

Figure 2. The differential regulation of HMG CoA reductase activities in sterologenic and tumor cells, illustrated with greatly simplified mevalonate pathway. Reductase activity in sterologenic cells (A) is subject to complex feedback regulation signaled by the sterol end product, cholesterol, and by a nonsterol metabolite diverted from the pathway, tentatively farnesol, with sterol-mediated transcriptional feedback being the primary regulatory mechanism. A residual reductase activity maintains pools of essential mevalonate-derived intermediates in the presence of a saturating concentration of cholesterol. The fine-tuning of HMG CoA reductase activity *via* the suppression of reductase mRNA translation and enhancement of reductase degradation is triggered when a metabolite is diverted from one of the pools, tentatively the farnesyl diphosphate pool, and by exogenous isoprenoids. The elevated and sterol feedback-resistant HMG CoA reductase activity in tumor cells (B) maintains the pools of essential mevalonate-derived intermediates required for the malignant proliferation of cells. In the absence of sterol feedback regulation, the secondary regulatory activities signaled by the diverted metabolite and by exogenous isoprenoids modulate reductase activity. The differential regulation of reductase activity, we suggest, underlies the isoprenoid-mediated suppression of proliferation specific for tumors. The arrows represent the relative impact of regulatory actions on reductase activity in normal and tumor cells.

activation might involve the binding of a coregulator protein, Red 25, within the *SREBP* site (157). Membrane-bound *SREBPs* are released from a cholesterol-depleted endoplasmic reticulum by a two-step proteolytic cascade; the mature NH_2-terminal fragments of the *SREBPs* enter the nucleus, where they upregulate the transcription of genes encoding sterologenic activities. *SREBP-1c*, a third member of the *SREBP* family, preferentially enhances transcription of genes required for fatty acid synthesis (158). *SREBP-1c* is a much weaker activator of the transcription genes required for sterologenesis than is *SREBP-1a* or *SREBP-2*. In liver there is an abundance of *SREBP-1c* relative to *SREBP-1a* (159). The total amount of *SREBP-2* increases in response to sterol depletion (155, 160) and decreases in response to sterols (158).

The post-transcriptional 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. Current studies conclude that farnesol (161–163) is the mevalonate-derived nonsterol mediator of HMG CoA reductase degradation. Farnesol (161–163), farnesol derivatives (farnesyl acetate, Ref. 164; ethyl farnesyl ether, Ref. 164; and farnesol homologs [tocotrienols], Ref. 165), and geranylated tocol analogues (166) accelerate the degradation of HMG CoA reductase by a nonlysosomal cysteine protease. Differences in HMG CoA reductase protein levels that cannot be attributed solely to a change in HMG CoA reductase mRNA level or in HMG CoA reductase degradation support the concept that an endogenous mevalonate-derived product modulates reductase synthesis at the translational level (167). Tocotrienol (165) and farnesol (167) decrease the efficiency of HMG CoA reductase mRNA translation. Geraniol suppresses HMG CoA reductase activity by suppressing both HMG CoA reductase transcription and mRNA translation (145). Limonene (145, 168) and perillyl alcohol (145) decrease reductase mass by decreasing the efficiency of HMG CoA reductase mRNA translation (145). The impact of the cyclic monoterpenes on HMG CoA reductase activity might be secondary to changes in cytosolic farnesol, the presumptive endogenous mevalonate-derived post-transcriptional modulator of reductase activity (Fig. 1, reviewed in Ref. 1; Refs. 161, 169).

In summation, farnesol or another isoprenoid distal to farnesol diphosphate in the sterologenic pathway mediates the post-transcriptional control of HMG CoA reductase synthesis and degradation (reviewed in Ref. 1). The exogenous mevalonate-derived products delineated in the preceding section trigger, with varying degree of potency, the post-transcriptional regulatory actions signaled by farnesol of endogenous origin.

Regulation of Tumor HMG CoA Reductase: Target for Tumor-Specific Isoprenoids

HMG CoA reductase activity in tumor cells, however, is elevated and dysregulated. Reductase activities in leukemia cells (170, 171) and lung carcinoma cells (172) are 3–8-fold and 2-fold higher, respectively, than those of normal cells. A higher portion of reductase activity is concomitantly directed toward the syntheses of nonsterol intermediates (Fig. 2B; Ref. 172). Coincidentally, HMG CoA reductase activity in tumor cells is, albeit resistant to sterol-mediated feedback regulation, severalfold more sensitive than normal cells to isoprenoid-mediated post-transcriptional downregulation (165, reviewed in Ref. 1), and isoprenoids preferentially impact the growth of malignant cells (65–74). When evaluated in animals, the levels of isoprenoids required to suppress tumor growth have no impact on tumor-free animals other than modestly lowering their serum cholesterol levels (63, reviewed in Ref. 1). The differential impacts on growth of malignant cells compared to normal cells, noted with isoprenoids but not lovastatin (Table 1), offer a clue, we suggest, to the mechanism underlying the numerous isoprenoid-mediated

activities recorded in Tables 1 and 2. The uncoupling of HMG CoA reductase activity from sterol-mediated feedback regulation, a fundamental lesion of malignant cells, permits the synthesis of the nonsterol products of the mevalonate pathway essential for cell survival in a sterol-rich environment (reviewed in Ref. 1). Post-transcriptional actions triggered by the aforementioned classes of isoprenoids and minor regulatory impacts secondary to the sterol-mediated feedback regulation in normal cells have major regulatory impacts on tumor HMG CoA reductase activity in the absence of sterol feedback. These impacts are enhanced by the elevated reductase activity and the increased demand for nonsterol products in tumor cells. Consequently, isoprenoids alter the proliferative and differentiation characteristics of a broad variety of malignant mammalian cell lines (65–74).

The dysregulation of reductase activity in tumor cells (Fig. 2B) may be explained by our recent findings of several-fold higher copies of HMG CoA reductase mRNA in human colon tumor (Caco2) and leukemic (CEM) cells compared to levels present in normal colon cells (CCD-18) and normal human lymphocytes (173). Findings that HMG CoA reductase promoter activity was 3-fold higher in Caco2 tumor cells than in CCD-18 normal cells suggest that the differential binding of transcription factor(s) on the reductase promoter is responsible for the attenuation of the normal sterol-mediated regulation of reductase activity (173). Observations of the inverse correlation between DNA methylation and gene expression levels (174); of the hypomethylation of proto-oncogenes, Ha-*ras*, Ki-*ras*, c-*fos*, c-*myc*, *erb*-A1, and *bcl*-2 in human leukemias and liver tumors (174), and of the hypomethylation of Ha-*ras*, c-*fos*, c-*myc*, and HMG CoA reductase in nodules in livers of rats exposed to diverse carcinogens (175, 176) led us to examine the methylation status of the promoter regions of HMG CoA reductase genes from solid tumor, leukemic, and normal cells. On finding that the reductase promoter sequences in both normal and malignant cells were hypomethylated, we concluded that an aberrant methylation pattern does not alter the binding of transcription factors to the promoter region (173).

Investigations of the upregulation of lipogenic activities in tumor tissues support another hypothesis, namely, that the transcription factor *SREBP-1a* is overexpressed in tumor tissues. Contrary to the relative abundance of *SREBP-1c* to *SREBP-1a* in liver tissue (159), in cultured embryonic and hepatoma cells the expression of *SREBP-1a* is reported to exceed that of *SREBP-1c* (156, 160, 177, 178). Although growth factor signaling *via* activation of PI3 kinase and MAP kinase increases *SREBP-1c* levels, such signals have no impact on the levels of *SREBP-1a* (179–182).

The aberrant HMG CoA reductase activity in tumor cells might reflect the overexpression of the *SREBP-1a* transcription factor. *SREBP-1* expressed in tumors (178) and in livers of mice overexpressing mature *SREBP-1a* (183) does not respond to sterol-mediated downregulation. In another approach, most embryonic mice lacking the *SREBP-1a* gene died *in utero* around Day 11. Consistent with the finding that mevalonate-derived products other than sterols are essential, surviving mice lacking the *SREBP-1a* gene had elevated hepatic *SREBP-2* mRNA, elevated mature *SREBP-2* in the nuclei, and elevated HMG CoA mRNA (184).

Lovastatin-mediated cholesterol depletion in cultured cells initiates the proteolytic cascade leading to the elevation of nuclear *SREBP-1a* and *SREBP-2* and to increased HMG CoA reductase mRNA transcription and translation with the resultant increase in HMG CoA reductase mass (185). Perillyl alcohol (145) and γ-tocotrienol (165) lower HMG CoA reductase mass in cultured cells by decreasing translational efficiency; γ-tocotrienol also increases reductase degradation (165). Other studies show that the actions triggered by isoprenoids block the statin-induced increase in HMG CoA reductase mass (172, 186, 187).

The metabolic error underlying the widely reported upregulation of HMG CoA reductase activity in tumors (reviewed in Ref. 1) remains to be delineated (Fig. 2B). Potential disturbances include the aforementioned increase in *SREBP-1a* relative to *SREBP-1c* in rapidly proliferating cells (156, 160, 177, 178). A mutation in the *SREBP* cleavage-activating protein (SCAP), a G→A transition at codon 443 of SCAP changing aspartic acid to asparagine, enhances its cleavage-stimulating activity, thereby rendering HMG CoA reductase resistant to sterol regulation (188). Although the basis of the dysregulated activity remains unresolved, the activity retains high sensitivity to isoprenoid-mediated post-transcriptional downregulation (165, 172, 186).

Recapping the foregoing discussion, lovastatin and isoprenoids suppress HMG CoA reductase activity, the former by competitive inhibition, the latter by post-transcriptional downregulation of the enzyme. Lovastatin induces the upregulation of Ras, Rap1a, RhoA, and RhoB (111, 122, 123, 189). The mechanisms underlying this upregulation, like that of HMG CoA reductase (2, 155), include increased mRNA synthesis, increased protein synthesis, and decreased protein degradation. The parallel impact of lovastatin on the upregulation of the small G proteins and HMG CoA reductase likely traces to depletions of the pools of farnesyl diphosphate, geranylgeranyl diphosphate, and cholesterol as a consequence of the competitive inhibition of HMG CoA reductase activity. Isoprenoids lower cellular levels of small G proteins (111, 116, 190) likely as a consequence of the post-transcriptional actions previously shown to lower cellular HMG CoA reductase (145, 165). Finally, isoprenoids attenuate the lovastatin-mediated upregulation of small G proteins and HMG CoA reductase (111). The latter finding casts doubt on the interpretation of results of prior studies, namely, that perillyl alcohol inhibits farnesyl protein transferase activity (111).

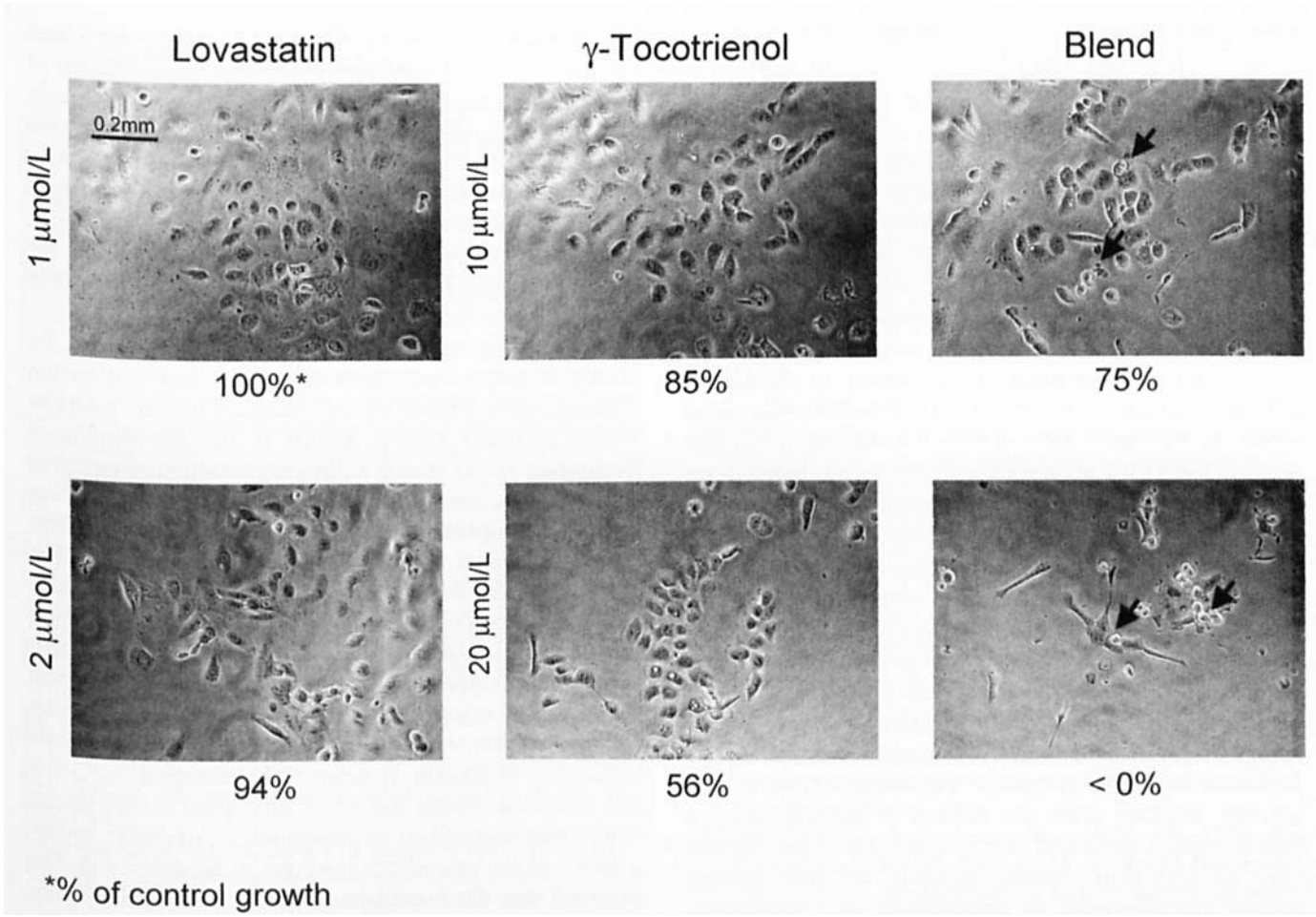

Figure 3. Synergistic impact of lovastatin and γ-tocotrienol on the proliferation of human DU145 prostate carcinoma cells. DU145 cells were inoculated at 1000 cells/well and cultured in 96-well plates. After a 24-hr incubation the medium was replaced with media containing lovastatin (1 or 2 μ*M*), γ-tocotrienol (10 or 20 μ*M*), or blends of the two agents. After additional 72-hr incubation, photomicrographs of the cells were taken and the cell population measured using the CellTiter 96 Aqueous One Solution (Promega, Madison, WI) procedure. Percentages of control growth are listed below the photomicrographs. Markers of apoptosis, nuclear condensation, membrane blebbing and differentiation, and elongation are noted with the arrows in the blend-treated cells.

Interaction of Lovastatin and Isoprenoids: A Potential Therapeutic Approach

Consequent to its nondiscriminant inhibition of reductase activity, lovastatin is a potent but nondiscriminant suppressor of cell proliferation, manifested by G1 phase arrest and apoptotic cell death. Diverse classes of isoprenoids suppress cell proliferation, albeit with lower potency than lovastatin, but with discrimination for malignant cells overexpressing HMG CoA reductase (65–74, 165). The IC_{50}value for β-ionone in suppressing normal colon CCD-18Co cell growth is 3-fold higher than that for Caco2 colon tumor cells (68), a disparity mirroring the difference in HMG CoA reductase mRNA levels between the two cell lines (173).

Tumor cell lines vary in sensitivity to lovastatin and various isoprenoids. The human DU145 prostate carcinoma cell line is relatively resistant to lovastatin (IC_{50} 22 μ*M*), γ-tocotrienol (IC_{50} 23 μ*M*), and perillyl alcohol (IC_{50} 430 μ*M*; Mo, unpublished data). The differential between γ-tocotrienol and perillyl alcohol is perhaps explained by findings that the tocotrienols act at both translational and post-translational levels (165), whereas perillyl alcohol acts only at the translational level (145). These observations suggest that the impacts of lovastatin and a tumor-targeted isoprenoid on tumor cell proliferation might be additive. The potential for gaining a therapeutic advantage by combining the two classes of agents has been demonstrated using the resistant DU145 cells.

Physiologically attainable serum concentrations of lovastatin (1 or 2 μ*M*) failed to suppress the proliferation of DU145 cells (Fig. 3). Incubation with a physiologically attainable serum level of γ-tocotrienol (10 μ*M*) yielded 15% suppression of growth, doubling the concentration of γ-tocotrienol yielded a 44% suppression (Fig. 3). Various lovastatin/γ-tocotrienol blends suppressed the growth of DU145 cells to a greater extent than predicted by the individual actions: a 1/10 (e.g., 1 μmol lovastatin, 10 μmol γ-tocotrienol/L) blend suppressed growth by 25% (Fig. 3), a

2/10 blend by 60% (not shown), a 1/20 blend by 85% (not shown), and a 2/20 blend by 100% (Fig. 3). Markers of apoptosis, nuclear condensation, and membrane blebbing and differentiation, elongation, are noted on the photomicrographs. This demonstration of the synergistic impact of lovastatin and γ-tocotrienol, also shown in human LNCaP prostate carcinoma cells (Mo, unpublished data) and murine B16 melanoma cells (68), lends support to our postulate that starvation of mevalonate-derived intermediates imposed by combining a potent but nondiscriminant inhibitor of HMG CoA reductase activity, lovastatin, with a less potent but discriminant suppressor, γ-tocotrienol, affords a therapeutic advantage beyond that of either agent alone. In this blend the γ-tocotrienol–mediated reductase degradation (165) counteracts the lovastatin-induced increase in reductase mass (155). Substituting γ-tocotrienol with a more potent isoprenoid derivative with a lower IC_{50} value (93) may offer further advantage. Despite its dramatic effects shown in preclinical studies, lovastatin at high doses leads to substantial toxicity in clinical studies, as outlined in the clinical studies section of this review. Dose-limiting toxicities also prevent the therapeutic application of other nondiscriminant suppressors of mevalonate pathway activities, namely, the mevalonate-pyrophosphate decarboxylase inhibitors and farnesyl protein transferase inhibitors. The synergy resulting from the adjunctive application of a tumor-targeted isoprenoid may markedly lower the effective dose of lovastatin, thereby avoiding the dose-limiting toxicity that precludes its development as a chemotherapeutic agent.

Future Directions

The primary event in the widely reported isoprenoid-mediated arrest of tumor cells in the G1 phase of the cell cycle and initiation of apoptosis remains to be delineated. Many of the reports of the isoprenoids's impact on the expression of genes, activation of signaling pathways required for the entry of cells into the S phase, modulation of the expression of genes, and activation of activities associated with apoptosis reflect single observations and await confirmation, whereas the parallel impacts triggered by lovastatin are widely confirmed.

The above discussion emphasized biological responses to isoprenoids and isoprenoid derivatives shown in preclinical evaluations to have chemotherapeutic potential. Reviews of experimental (1, 63, 191) and population (192) studies suggest that dietary isoprenoids provide a measure of chemoprevention (1, 63, 191). A recent study provided a list of 179 volatile isoprenoid constituents of members of seven plant families prominent in Western diets. Of these, 41 were screened for tumor-suppressive activity *in vitro;* IC_{50} values ranged from 28 to over 1000 μ*M*. Findings that blends of isoprenoids suppressed growth of murine B16 melanoma and human HL60 leukemic cells with efficacies equal to the sum of the individual impacts suggest that the cancer-protective property of fruits, vegetables, and related products is in part conferred by the cumulative impact of volatile isoprenoid constituents. Quantitation of the dietary intake of individual isoprenoids provided by fruits and vegetables is precluded by variety and variations in growing conditions, maturity, processing, and postharvest storage, as well as the undefined number of constituent isoprenoids. Studies of isoprenoid metabolism may lead to the identification of a biological marker of intake and ultimately a means for predicting cancer risk (64).

The mevalonate pathway remains a viable target for chemoprevention and chemotherapy in that it provides essential intermediates for a plethora of cellular functions related to tumor growth. Efforts in the development of therapeutic agents aiming at the enzyme activities related to the pathway, namely, HMG CoA reductase, mevalonic acid–pyrophosphate decarboxylase, and farnesyl protein transferase, have not yet borne fruit in the clinic. The nondiscriminant inhibitory strategies reviewed herein have extensive impact on the expression of growth-related genes, protein prenylation, diacylglycerol signaling, and mevalonate synthesis; their dose-limiting toxicities interfere with their clinical application. HMG CoA reductase, the rate-limiting activity, remains an epitome target because of its differential regulation in tumor and sterologenic cells (1). The molecular events that occur subsequent to mevalonate deprivation imposed by an isoprenoid, by lovastatin, and by a blend of the two agents have yet to be delineated. The proposal that the two-agent blend offers a novel chemopreventive and/or chemotherapeutic approach free of the dose-limiting toxicity associated with high-dose lovastatin requires preclinical evaluation. We suggest that modulating the regulation rather than directly inhibiting the activity of HMG CoA reductase avoids the toxicities imposed by the nondiscriminant inhibitors.

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