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Review

Dietary factors and the regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase: Implications for breast cancer development

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A role for mevalonate in cancer development has long been suggested by findings that 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity is elevated in malignant cells. Increased synthesis of mevalonate and mevalonate-derived nonsterol isoprenoids supports increased cell proliferation through the activation of growth-regulatory proteins and oncoproteins, and by promoting DNA synthesis. We have recently shown that mevalonate promotes the growth of human breast cancer cells both in culture and as tumors grown in nude mice. Inhibiting mevalonate synthesis, therefore, may be an effective strategy to impair the growth of malignant breast cells. Several dietary compounds with known anti-cancer effects are also reported to inhibit HMG-CoA reductase activity. Here, we review evidence suggesting that inhibition of mevalonate synthesis may mediate the protective effects of cholesterol, plant isoprenoids, genistein, and long-chain *n*-3 polyunsaturated fatty acids (PUFAs) on experimental breast cancer.

Keywords: Cholesterol / Dietary isoprenoids / Genistein / 3-Hydroxy-3-methylglutaryl coenzyme A reductase / *n*-3 Polyunsaturated fatty acids / Review

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Contents

1 Introduction

3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase is the rate-limiting enzyme in cholesterol biosynthesis that catalyzes the formation of mevalonate from HMG-CoA (Fig. 1) [1]. Mevalonate is also a precursor of the nonsterol isoprenoids that function in diverse cellular processes [1]. A role for mevalonate in cancer development has long been suggested by findings that HMG-CoA reductase activity is increased in diverse tumor types, including breast [2], lung [3], leukemia and lymphoma [4], and hepatocellular carcinoma [5], and that feedback regulation of reductase by cholesterol is deficient in malignant cells [2, 6]. Interest in the mevalonate pathway as a target for cancer chemoprevention and chemotherapy has increased with the discovery of specific growth-regulatory roles for mevalonate and mevalonate-derived isoprenoids in malignant cells. Mevalonate is required for DNA synthesis and cell proliferation, independent of cellular requirements for cholesterol [7]. It has been shown to increase the proliferation of human breast cancer cells in culture [8, 9] and to promote the growth of these cells as tumors in athymic nude mice [8]. The nonsterol isoprenoids farnesyl pyrophosphate and geranylgeranyl pyrophosphate are required for prenylation, the process by which proteins are covalently modified by attachment of a highly lipophilic polyisoprene unit allowing for targeting to lipophilic regions of the cell [10, 11]. Prenylation may be of particular importance for the development of some tumors, since it is required for the activation of

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Abbreviations: DHA, docosahexaenoic acid; **DMBA**, 7,12-dimethylbenzanthracene; **EPA**, eicosapentaenoic acid; **HMG-CoA**, 3-hydroxy-3-methylglutaryl coenzyme A; **LDL**, low-density lipoproteins; **PUFAs**, polyunsaturated fatty acids

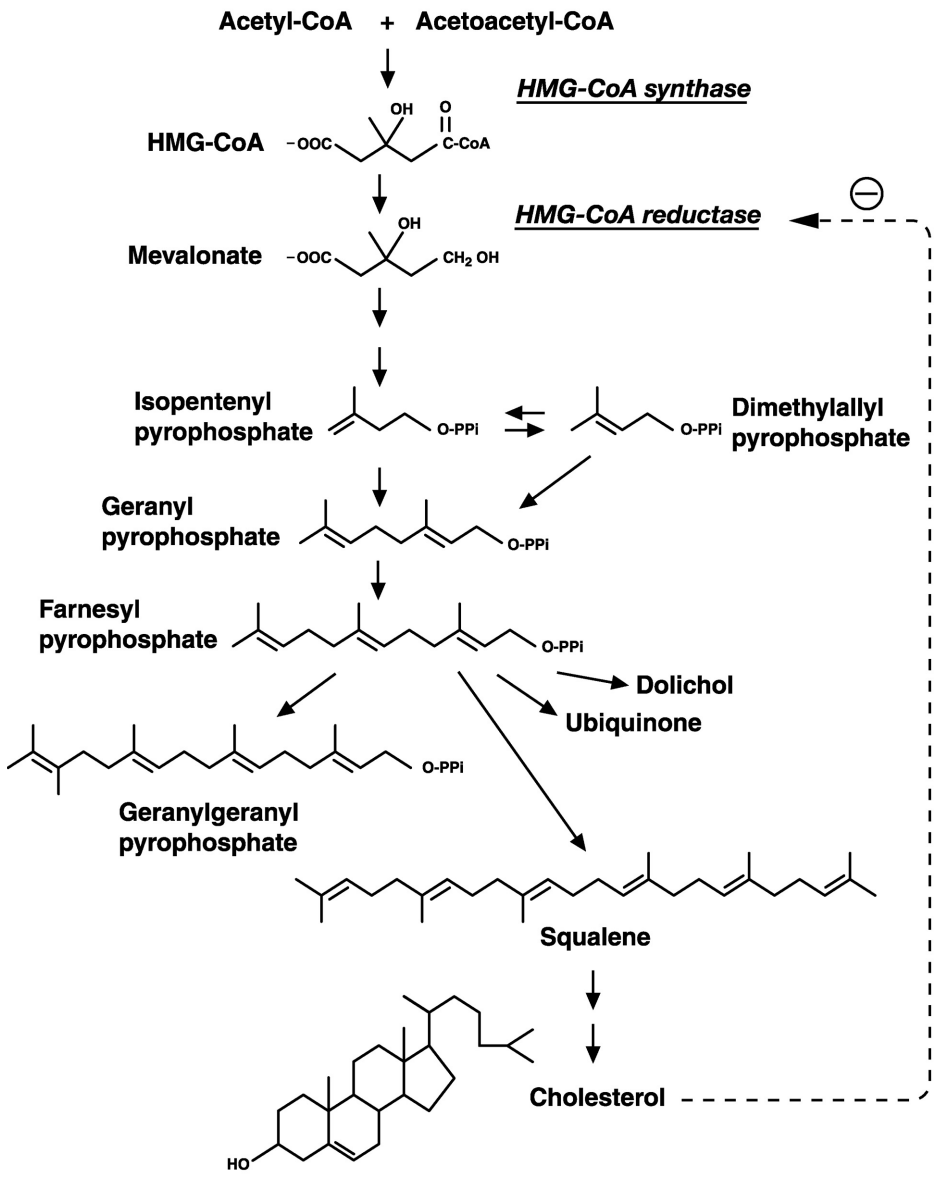

Figure 1. The mevalonate pathway.

growth regulatory proteins [12, 13] and transforming oncoproteins such as Ras and Rho [14, 15]. Dolichyl pyrophosphate is also utilized to modify proteins in a process that is required for *N*-linked glycosylation [16, 17]. Like prenylation, dolichylation and subsequent glycosylation are necessary for the proper subcellular localization of some proteins, including the insulin-like growth factor-1 receptor that plays a role in maintenance of the transformed phenotype in several cancer cell lines [18, 19].

Much of our understanding of the role of mevalonate in the growth of malignant cells comes from studies that have employed statin drugs, a group of compounds that competitively inhibit HMG-CoA reductase resulting in mevalonate depletion. From these studies we know that impaired mevalonate synthesis arrests the growth of cells [7, 20], while prolonged mevalonate depletion causes apoptosis and cell death [21]. These growth-inhibitory effects, however, are evident in both normal and neoplastic cells at similar statin concentrations, suggesting that toxicity may preclude the use of these drugs in anti-cancer therapy [20]. While studies in rodents have, nevertheless, suggested that statins may be effective for cancer chemoprevention and chemotherapy [22–24], the appearance of myotoxicity has, indeed, proved to be dose-limiting for their use in humans [25].

Several dietary components are also known to inhibit HMG-CoA reductase activity and mevalonate synthesis. Unlike statins, these compounds are often without any reported adverse effects, even at levels substantially above

those typically consumed in the human diet. Here, we review evidence suggesting that inhibition of mevalonate synthesis may mediate the protective effects of cholesterol, plant isoprenoids, genistein, and long-chain *n*-3 polyunsaturated fatty acids (PUFAs) on experimental breast cancer.

2 Cholesterol

In most cells, cholesterol is derived both from endogenous synthesis *via* the mevalonate pathway, and from the uptake of circulating cholesterol-rich low-density lipoproteins (LDLs) that are internalized *via* the LDL receptor [1]. A rise in serum cholesterol concentration that increases intracellular cholesterol levels represses mevalonate synthesis through feedback regulation of HMG-CoA reductase [1, 26]. Conversely, a fall in serum cholesterol concentration stimulates mevalonate synthesis [26]. Since mevalonate or a mevalonate-derived nonsterol metabolite is required for cell proliferation [7], regulation of HMG-CoA reductase in preneoplastic cells by serum cholesterol may influence the development of breast tumors.

In human studies, a link between low serum cholesterol levels and increased risk of cancer in general has long been recognized [27]. No clear pattern of risk has emerged, however, with respect to breast cancer in particular. Although some studies have found an inverse association between serum cholesterol concentration and breast cancer risk [27 – 29], interpretation of this data with respect to causality is confounded by the strong association of dietary cholesterol levels with intakes of other dietary factors, such as fats. A further complication is that humans vary in their response to increases in dietary cholesterol – some individuals show a marked increase in serum cholesterol, while others show no such response, or, in some cases, actually show reduced serum levels [30, 31].

A stronger relationship between serum cholesterol and the growth of malignant tumors of the breast epithelium has been demonstrated in rodent carcinogenesis studies. We have shown previously that a diet containing cholesterol significantly inhibits the development of mammary tumors in rats treated with the mammary carcinogen *N*-methyl-*N*-nitrosourea [32] or 7,12-dimethylbenzanthracene (DMBA) [2, 32] compared to those fed a cholesterol-free diet. Cholesterol-fed rats had elevated serum cholesterol levels that significantly suppressed HMG-CoA reductase activity in mammary glands [2]. We postulated that feedback inhibition of mevalonate synthesis in preneoplastic cells and early neoplastic lesions mediated the protective effect of dietary cholesterol on mammary carcinogenesis. In an earlier experiment, Melhem *et al.* [33] demonstrated the inverse of this effect when serum cholesterol levels were lowered. In rats initiated with DMBA, then treated with the cholesterol-lowering agent cholestyramine, a 5-fold increased incidence of malignant mammary tumors was found compared to control rats that did not receive the agent [33]. Cholestyramine is a bile acid-binding resin that is unabsorbed. Therefore, any effects of this compound on carcinogenesis at sites distal to the intestinal lumen must be mediated indirectly. It was hypothesized that stimulation of mevalonate synthesis provided the mitogenic signal to transformed cells that resulted in increased mammary tumor development [33]. In a subsequent study cholestyramine was, indeed, shown to cause an induction of *de novo* cholesterol synthesis in the mammary glands of rats that was stimulated by the fall in serum cholesterol concentration produced by the resin [34].

These studies in rodents demonstrate that changes in serum cholesterol concentration influence mammary tumor development. Despite the potential chemopreventive benefit, it would clearly be inappropriate to recommend consumption of diets that increase serum cholesterol levels, given the association of elevated serum LDL concentration with development of cardiovascular disease [31]. Other dietary compounds with effects on HMG-CoA reductase, however, have been identified that offer both chemo-protective and cardio-protective benefits.

3 Plant isoprenoids

Plant isoprenoids are non-nutritive compounds synthesized in plants from intermediates of mevalonate metabolism [35 – 37]. The principle dietary sources of these compounds are vegetables, grains, and essential oils of fruits [35]. There are estimated to be over 20 000 different compounds in this group that include both the pure and mixed isoprenoids [36]. Pure isoprenoids differ in structural complexity, but consist only of multiples of the basic 5-carbon isoprene unit ($1\times$) and include the monoterpenes ($2\times$), sesquiterpenes ($3\times$), diterpenes ($4\times$), triterpenes ($6\times$), tetraterpenes ($8\times$), and polyterpenes ($n\times$). The mixed isoprenoids are compounds in which only a part of the molecule is derived from the mevalonate pathway, and these include the prenylated coumarins, flavones, flavonols, isoflavones, chalcones, quinones, and chromanols [36].

The anti-carcinogenic activity of plant isoprenoids has been tested in rodent mammary carcinogenesis experiments. The monoterpene *d*-limonene, which makes up greater than 90% of orange peel oil, has been shown to decrease tumor multiplicity, increase tumor latency, decrease tumor yield, and induce the frank regression of tumors when fed to rats at various time points during the initiation and promotion/progression stages of mammary carcinogenesis [38 – 41]. Carveol, uroterpenol, and sobrerol, three hydroxylated derivatives of *d*-limonene, have also been shown to prolong tumor latency and decrease tumor yield when fed to rats

during the initiation stage of mammary carcinogenesis [39]. Furthermore, the monocyclic monoterpene menthol, the tricyclic monoterpene β-ionone, and the acyclic monoterpene geraniol were all shown to inhibit rat mammary carcinogenesis when fed to animals from two weeks prior to initiation until study completion [42]. Plant isoprenoids also suppress the proliferation of human breast cancer cells in culture. β-Ionone [43, 44] and geraniol [44] have been shown to inhibit the growth of MCF-7 cells, while perillyl alcohol and perillic acid were demonstrated to significantly inhibit the growth of T-47D, MCF-7, and MDA-MB-231 cells [45]. Similarly, a significant inhibitory effect of perillyl alcohol has been found on the growth of murine mammary adenocarcinoma cells [46]. Moreover, plant isoprenoids appear to inhibit the proliferation of breast cancer cells with a greater potency than normal mammary epithelial cells [44], suggesting that they may have promise for use as adjuvant chemotherapeutic agents. Indeed, limonene, perillic acid, and perillyl alcohol have been tested in clinical trials of late-stage breast cancer patients, and efforts in this area are ongoing [47–49].

Plant isoprenoids have been hypothesized in a number of reports to mediate their cancer chemopreventive effects through inhibition of HMG-CoA reductase activity and mevalonate synthesis [35–37, 50–52]. Pure and mixed plant isoprenoids have been found to inhibit mevalonate synthesis in cultured hamster kidney [53] and ovary cells [54], macrophages [55], in rat [56–58] and avian liver [50], and in the well-differentiated human hepatocellular carcinoma cell line HepG2 [59]. Inhibition of HMG-CoA reductase occurs primarily at a post-transcriptional level [58], and is mediated by increased degradation of the enzyme [57, 59] as well as decreased mRNA translational efficiency [53], resulting in decreased enzyme synthesis [53, 57, 59]. Inhibition of mevalonate synthesis may, therefore, mediate the chemopreventive effects of dietary isoprenoids in rodent mammary carcinogenesis and the anti-proliferative effects of these compounds in cell culture. Indeed, Elson and colleagues [50] have reported that the potency with which a plant isoprenoid suppresses hepatic reductase is predictive of its ability to inhibit the development of mammary tumors in rats. Few studies, however, have directly investigated the regulation of HMG-CoA reductase by these compounds in malignant cells or tissues of extra-hepatic origin. In MCF-7 human mammary adenocarcinoma cells, an oxidation product of β-carotene has been shown to inhibit cholesterol synthesis from acetate [60]. Mevalonate rescued the corresponding inhibitory effects of this compound on cell proliferation. In this study HMG-CoA reductase activity was not directly measured and inhibition of enzymatic steps following mevalonate synthesis, therefore, may have mediated growth inhibition. Recently, we measured the effects of geraniol and β-ionone on the growth of MCF-7 cells and their ability to inhibit HMG-CoA reductase in these cells [44]. Although both compounds inhibited cell growth, significant inhibition of reductase activity was demonstrated only by geraniol. It is clear, therefore, that β-ionone does not inhibit MCF-7 cell growth *via* a reduction of mevalonate synthesis. Effects of geraniol on proliferation and reductase activity in this cell line, however, were closely correlated, suggesting that this relationship may be causal. To determine whether depletion of mevalonate mediated the anti-proliferative effects of geraniol in MCF-7 cells, we tested the ability of exogenous mevalonate to restore growth. Surprisingly, mevalonate had no significant effect on the proliferation of cells that were growth-inhibited by geraniol. Our studies indicated, therefore, that growth inhibition of MCF-7 cells by both β-ionone and geraniol is mediated entirely through a mechanism that is independent of HMG-CoA reductase regulation.

These findings have implications for our understanding of the anti-proliferative effect of dietary isoprenoids in malignant cells. Caution should be exercised, however, in extrapolating results from this study to other experimental models. Although β-ionone did not inhibit HMG-CoA reductase in MCF-7 cells, it has been shown previously to inhibit this enzyme in normal liver [50]. This discrepancy may result from differences in the tissues examined but, alternatively, it could have been caused by differences arising during malignant transformation. Regulation of HMG-CoA reductase activity by β-ionone may, therefore, be intact in normal and preneoplastic mammary epithelium, and inhibition of mevalonate synthesis in preneoplastic cells may, indeed, mediate the inhibitory effect of this isoprenoid on rodent mammary carcinogenesis. Furthermore, regulation of mevalonate synthesis by dietary isoprenoids in preneoplastic cells may result in a greater growth-inhibitory effect than is evident in fully transformed, highly malignant cells. Additional studies are clearly required to elucidate whether modulation of mevalonate synthesis mediates chemoprevention of mammary carcinogenesis by dietary isoprenoids.

4 Genistein

Epidemiological studies have suggested that consumption of the soy isoflavone genistein confers a protective effect on the development of breast cancer [61–63]. Genistein is a weak estrogen that acts as a competitive inhibitor of more potent endogenous estrogens. In young nude mice with relatively high levels of endogenous estrogens, dietary genistein inhibits the growth of breast tumors derived from estrogen-dependent MCF-7 human breast cancer cells [64], but does not inhibit the growth of tumors derived from estrogen-independent MDA-MB-231 human breast cancer cells [65]. In cell culture, however, most human breast cancer cell lines, including both estrogen-dependent and estrogen-independent lines, are growth-inhibited by genistein at con-

centrations as low as 1 μM [65 – 68]. The anti-estrogenic action of this compound, therefore, does not entirely account for its growth inhibitory effects.

Recent studies suggest that inhibition of mevalonate synthesis through regulation of HMG-CoA reductase activity may contribute to the chemoprotective effects of genistein. Genistein is a competitive inhibitor of recombinant HMG-CoA reductase activity with a K_i of 27.7 μM [69]. Genistein has also been shown to inhibit cholesterol synthesis in cultured HepG2 cells [70]. Impaired cholesterogenesis in this study, however, was associated with an increase in HMG-CoA reductase mRNA expression, which likely indicates a compensatory response to competitive inhibition. Enzyme levels or activity were not measured. We have recently examined the regulation of HMG-CoA reductase by genistein in MCF-7 human breast cancer cells and found significant, concentration-dependent inhibition of activity that resulted from a decrease in enzyme level [71]. Unlike effects observed in HepG2 cells, however, genistein had no effect on mRNA expression. Determination of the role played by inhibition of mevalonate synthesis in mediating the chemopreventive effects of genistein in mammary carcinogenesis and breast cancer cell growth will require further study.

5 *n*-3 PUFAs

The long-chain *n*-3 PUFAs docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) inhibit the growth of both estrogen-dependent and estrogen-independent human breast cancer cell lines [72 – 80] at concentrations that do not have any anti-proliferative effect on normal human breast cells or fibroblasts [74]. Conversely, the dietary long-chain *n*-6 PUFAs linoleic acid and arachidonic acid stimulate the growth of human breast cancer cells [81, 82]. Although somewhat controversial, long-chain *n*-3 and *n*-6 PUFAs also appear to have opposing effects on breast cancer risk [83, 84], and on the development of mammary cancer in rats [85, 86].

Mechanisms mediating the differential effects of *n*-3 and *n*-6 PUFAs on breast cancer growth are complex and likely numerous [87]. Despite decades of research in this area, the exact mechanism underlying the cancer protective effects of EPA and DHA remains unclear [77, 87]. There is evidence that EPA and DHA may inhibit breast cancer cell proliferation and mammary cancer development, at least in part, through inhibition of HMG-CoA reductase activity and mevalonate synthesis. Dietary fish oils, a rich source of EPA and DHA, inhibit hepatic HMG-CoA reductase activity in rats [88 – 91], mice [92], and rabbits [93], and also inhibit mammary gland reductase in rats [90] and mice [94]. EPA and DHA also inhibit reductase activity in rat liver when administered individually [95 – 98]. Feeding fish oils to mice has been shown to decrease hepatic HMG-CoA reductase gene transcription in mice [92, 99]. Long-chain *n*-3 PUFAs have been shown to inhibit HMG-CoA reductase activity in CaCo-2 colon cancer cells [98] and H35 hepatoma cells [100]. Recently, we found that EPA and DHA also inhibit reductase activity in MCF-7 human breast cancer cells [71]. Unlike effects reported in mouse liver [92, 99], however, we found no changes in reductase mRNA expression in this malignant cell line.

6 Conclusions

A number of dietary factors with established effects on the growth of malignant breast cells are known to regulate HMG-CoA reductase activity. Clearly, considerable efforts will be required to elucidate whether this relationship is, indeed, causal. Given current limitations in the use of competitive inhibitors of HMG-CoA reductase for cancer chemotherapy, identification of dietary compounds that mediate growth-inhibition through regulation of the mevalonate pathway may provide novel agents that will aid in the prevention and adjuvant treatment of breast cancer. Identification of dietary factors that enhance the synthesis of mevalonate in the mammary epithelium may also lead to strategies to reduce the risk of developing this disease. Continued investigation of the regulation of HMG-CoA reductase activity and mevalonate synthesis by dietary compounds is likely to yield information that has important implications for the control of breast, as well as other cancers.

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