Abrogation of Insulin-like Growth Factor-I (IGF-I) and Insulin Action by Mevalonic Acid Depletion

SYNERGY BETWEEN PROTEIN PRENYLATION AND RECEPTOR GLYCOSYLATION PATHWAYS*

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The vasculoprotective effects of hydroxy-3-methylglutaryl (HMG)-CoA reductase inhibitors (statins) correlate with cholesterol lowering. HMG-CoA reductase inhibitors also disrupt cellular processes by the depletion of isoprenoids and dolichol. Insulin and insulin-like growth factor (IGF) signaling appear particularly prone to such disruption as intracellular receptor processing requires dolichol for correct N-glycosylation, whereas downstream signaling through Ras requires the appropriate prenylation (farnesol). We determined how HMG-CoA reductase inhibition affected the mitogenic effects of IGF-I and metabolic actions of insulin in 3T3-L1 cells and examined the respective roles of receptor glycosylation and Ras prenylation. IGF-I- and insulin-induced proliferation was significantly reduced by all statins tested, although cerivastatin (10 μM) had the greatest effect (p < 0.005). Although inhibitors of Ras prenylation induced similar results (10 μM FTI-277 89% ± 7.4%, p < 0.01), the effect of HMG-CoA reductase inhibition could only be partially reversed by farnesyl pyrophosphate refeeding. Treatment with statins resulted in decreased membrane expression of receptors and accumulation of proreceptors, suggesting disruption of glycosylation-dependent cleavage. Glycosylation inhibitors inhibited IGF-I-induced proliferation (tunicamycin p < 0.005, castanospermine p < 0.01, deoxymannojirimycin p < 0.01). High concentrations of statin were necessary to impair insulin-mediated glucose uptake (300 μM = 33% ± 12% p < 0.05), and this process was not affected by farnesyl transferase inhibition. Glycosylation inhibitors mimicked the effect of statin treatment (tunicamycin p < 0.001, castanospermine p < 0.05, deoxymannojirimycin p < 0.05), and there was insulin proreceptor accumulation. These data imply that HMG-CoA reductase inhibitors disrupt IGF-I signaling by combined effects on Ras prenylation and IGF receptor glycosylation, whereas insulin signaling is only affected by disrupted receptor glycosylation.

HMG-CoA reductase inhibitors are widely and successfully used to treat cardiovascular disease (1). Although their benefits are largely attributed to low density lipoprotein cholesterol lowering, there is increasing interest in the pleiotropic effects of these agents. HMG-CoA reductase inhibitors (statins) reduce the formation of mevalonic acid, an early precursor in the biosynthesis of cholesterol (2). However, mevalonic acid depletion also results in decreased levels of dolichol and isoprenoids (3); dolichol is intimately involved in the process of N-linked glycosylation of membrane-targeted proteins, whereas isoprenoids are necessary for the prenylation, subsequent membrane anchoring, and activity of downstream growth factor signaling components such as Ras. Thus statins may exert additional effects through modulation of post-translational glycosylation and isoprenylation.

Insulin-like growth factor (IGF-I) and -II are polypeptides with significant structural homology to insulin. Consequently, they have acute anabolic effects on carbohydrate and protein metabolism, although importantly, they are also potent regulators of cellular replication, differentiation, and survival (4). We and others have recently found that abnormally low levels of IGF-I are associated with the premature development of type 2 diabetes (5, 6). Conversely, IGF-I is also known to promote many of the processes involved in the formation of atherosclerotic lesions and cardiovascular disease since IGF-I stimulates macrophage chemotaxis; endothelial cell migration (7); and vascular smooth muscle cell proliferation and migration (8). It also primes macrophages and monocytes for cytokine release (9). These studies clearly demonstrate that the IGF and insulin signaling systems are involved in the aetiopathogenesis of cardiovascular disease and diabetes.

Statins act via inhibition of hydroxy-3-methylglutaryl CoA reductase in the early stages of cholesterol biosynthesis. These agents are widely and successfully used to treat cardiovascular disease (1). The pleiotropic effects of these agents are also widely recognized, including a reduction in cardiovascular mortality and an improvement in insulin sensitivity in type 2 diabetes (10). The mechanisms underlying these beneficial effects are not fully understood, but it is becoming clear that the vascular effects of statins may be mediated via the IGF signaling system (11).

Statin treatment causes rapid and marked decreases in circulating IGF-I levels, and this reduction is associated with improvements in insulin sensitivity (12). This effect is not due to a direct effect on IGF-I production by the liver, as the levels of other serum proteins are unaffected (13). The decrease in IGF-I levels is likely to be a secondary effect of statin treatment, possibly due to decreased hepatic IGF-I production or increased clearance. This decrease in IGF-I levels may contribute to the reduction in cardiovascular mortality and improvement in insulin sensitivity observed with statin treatment, as IGF-I has been shown to be a risk factor for cardiovascular disease and type 2 diabetes (14).

IGF-I is a polypeptide that belongs to the insulin superfamily and plays a crucial role in the regulation of cell growth, proliferation, and differentiation. It is produced by many tissues and is implicated in a variety of physiological processes, including growth, development, and metabolism. IGF-I is a mitogenic factor for a wide range of cell types, including fibroblasts, endothelial cells, and smooth muscle cells. It also plays a key role in the regulation of glucose metabolism, as it promotes the uptake of glucose by muscle and adipose tissues (15).

The mechanism of action of IGF-I involves the activation of the IGF receptor (IGF-IR), a tyrosine kinase that is expressed on the cell surface. Upon binding of IGF-I, the IGF-IR undergoes autophosphorylation, which leads to the activation of downstream signaling pathways. One of the most important downstream targets of IGF-I signaling is the Ras/MAPK pathway, which is involved in the regulation of cell proliferation, survival, and differentiation. This pathway is activated by the binding of GDP to Ras, which triggers the exchange of GDP for GTP, leading to the activation of the Ras protein. Activated Ras then binds to and activates the mitogen-activated protein kinase (MAPK) cascade, which ultimately leads to the activation of transcription factors and the expression of target genes (16).

Recent studies have suggested that IGF-I signaling may also be regulated by the modification of proteins with isoprenoids. Isoprenoids are small lipids that are involved in the post-translational modification of proteins and are involved in a variety of cellular processes, including cell proliferation, differentiation, and survival. One of the main functions of isoprenoids is to modify proteins with farnesyl or geranylgeranyl groups, which are involved in the anchoring of proteins to the cell membrane and their interaction with other proteins. These modifications are catalyzed by farnesyl transferase and geranylgeranyl transferase enzymes, respectively.

The vasculoprotective effects of statins may be due, in part, to the inhibition of isoprenoid biosynthesis, which leads to a decrease in the production of farnesyl and geranylgeranyl groups. This decrease in isoprenoid levels may lead to a decrease in the modification of proteins with these groups, which may alter the function of IGF-I and insulin receptors. This may explain why statins can improve insulin sensitivity and reduce the risk of cardiovascular disease, even in the absence of changes in cholesterol levels.

In conclusion, statin treatment causes a decrease in circulating IGF-I levels, which may contribute to the reduction in cardiovascular mortality and improvement in insulin sensitivity observed with statin treatment. This effect is likely to be mediated via the IGF signaling system, and further research is needed to understand the mechanisms underlying this effect.
EXPERIMENTAL PROCEDURES

Materials—HMG-CoA reductase inhibitors were a kind gift of Bayer pharmaceuticals PLC and AstraZeneca PLC. Deoxymannojirimycin and the farnesyl transferase inhibitor FTI-277 were purchased from Calbiochem. Castanospermine was purchased from Alexis Corp. Farnesyl pyrophosphate was purchased from Sigma. Insulin and IGF receptor rabbit polyclonal antibodies for immunoprecipitation and Western blotting were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Protein Aagarose (2%) was purchased from CalBiochem.

Cell Culture—3T3-L1 cells (American Type Culture Collection, Manassas, VA) were grown in Dulbecco’s modified Eagle’s medium supplemented with 4 mM glutamine, 1 mM pyruvate, and 10% fetal calf serum. Cells overexpressing the human insulin receptor (3T3-IR) or human IGF receptor (NWTb3) were generously provided by Professor LeRoith (National Institutes of Health, Bethesda, MD) and cultured in 3T3-L1 medium containing 0.05 mg/ml geneticin. All cells were maintained at 37 °C in 5% CO2.

1H/3HThymidine Uptake Assay—3T3-L1 preadipocytes were incubated in serum-free medium with or without HMG-CoA reductase inhibitor for 24 h before the addition of IGF-I (10 ng/ml (1.3 nM)) or insulin (5 μM). 20 h later, [methyl-3H]thymidine was added to a final concentration of 0.05 μCi/ml, and after a further 4 h, cells were washed three times with ice-cold PBS before incubation with 10% trichloroacetic acid for 1 h at 4 °C. Cells were then solubilized with 0.1 M NaOH for assessment of total protein concentration and [3H]thymidine incorporation.

Differentiation of 3T3-L1 Preadipocytes—3T3-L1 preadipocytes were seeded at a density of 5 × 104 cells/ml (day 0) and grown until 2 days post-confluent (day 8). Differentiation was then induced by supplementing the growth medium with 2 μM insulin, 0.5 mM 3-isobutyl-1-methylxanthine, and 0.25 μM dexamethasone for 2 days. Triglyceride droplets could be observed in differentiated cells from day 14. In all experiments using differentiated 3T3-L1 adipocytes, more than 90% of the cells had acquired the adipocyte phenotype as demonstrated by Oil red O staining.

Glucose Uptake by Mature 3T3-L1 Adipocytes—3T3-L1 adipocytes were transferred to serum-free medium with or without inhibitor for 24 h and then placed in Hepes-buffered saline (150 mM NaCl, 5 mM KCl, 5 mM MgSO4, 1 mM CaCl2, 15 mM Hepes, pH 7.2) for 1 h before the addition of IGF-I or insulin (100 ng/ml) for 30 min. Glucose (2 mM 2-deoxy-glucose, 10 μCi/ml [3H]-2-deoxy-glucose) was added to each well, and after 15 min, uptake was stopped by the addition of 2 mM phloretin. Adipocytes were washed three times with ice-cold PBS and lysed in 0.1 M NaOH for analysis of protein content and [3H]-2-deoxy-glucose uptake.

Biotinylation of Cell Membranes—A confluent flask (75 cm2) of 3T3-L1 or NWTb3 cells was washed three times with cold PBS, incubated on ice for 15 min, and then treated with 5 mM of sodium metaperiodate (10 mM) for 30 min on ice. Cells were then washed three times with cold PBS and slowly agitated with 2 ml of 5 mM biotin hydrizade for 30 min before the addition of solid sodium cyanoborohydride (50 mM) and a further 30-min incubation. After washing three times with cold PBS, cells were scraped into radioimmune precipitation buffer (50 mM TrisCl, 1% Nonidet P-40, 0.25% deoxycholic acid, 150 mM NaCl, 1 mM EDTA) containing 1 mM sodium vanadate, 1 mM sodium fluoride, and protease inhibitors (CalBiochem).

Immunoprecipitation and Western Blotting of Insulin and IGF Receptors—Lysates of surface biotinylated cells (100 μg) were incubated with polyclonal anti-IGF receptor (Santa Cruz Biotechnology) or anti-insulin receptor (Santa Cruz Biotechnology) antibodies overnight at 6 °C and then with anti-rabbit IgG antibody (SacCel; IDS, Tyne & Wear, UK) for 1 h at room temperature. The immune complexes were pelleted by centrifugation, washed three times with PBS and then resuspended in reducing SDS loading buffer (0.125 mM TrisCl, pH 6.8, 2% SDS, 10% glycerol, 5% β-mercaptoethanol, 0.25% bromphenol blue). Immunoprecipitated proteins were electrophoresed on SDS/polyacrylamide (6%) gels, blotted onto nitrocellulose, and blocked (0.15 M NaCl, 1% Marvel). Biotinylated receptors were incubated with streptavidin linked to horseradish peroxidase and detected by ECL. Native receptors were incubated with either IGF I or insulin receptor rabbit polyclonal antibodies (SC-9038, SC-711, respectively, Santa Cruz Biotechnology) followed by anti-rabbit IgG linked to horseradish peroxidase (Sigma) and detected by ECL.

Determination of Apoptosis—Preadipocytes were either treated for 24 h with cerivastatin (0.3–300 nM) or treated for 24 h with 1 μM staurosporine. Following treatment, the medium was removed, and detached cells were pelleted by centrifugation. Attached cells were trypsinized and combined with detached cells. Cells were cytospan onto polylysine slides (Merck) and fixed in 3.7% paraformaldehyde for 10 min. Apoptosis was quantified by examining the nuclear morphology of cells stained with 4 μg/ml Hoechst 33258 (Molecular Probes). Each experiment was repeated three times, and in each experimental condition, more than 1000 single cells were scored for apoptosis.

Ras Localization—Confluent 3T3-L1 preadipocytes were treated with or without 300 nM cerivastatin for 24 h, washed three times with cold PBS, scraped into 50 mM HEPES plus protease inhibitor mixture (Calbiochem), and sonicated for 3 × 5 s at an amplitude of 20 μM. Intact cells were removed by centrifugation (500 × g, 5 min), the total protein content of the remaining supernatant was determined, and then equal amounts of protein (1 mg) were taken for membrane isolation by centrifugation at 190,000 × g for 45 min at 4 °C. Membrane pellets were resuspended in SDS loading buffer, electrophoresed on a SDS 10% polyacrylamide gel, blotted onto nitrocellulose, and probed with a rat monoclonal anti-Ras antibody. Bound antibody was detected by an anti-rat IgG antibody linked to horseradish peroxidase and ECL.

RESULTS

The Effect of HMG-CoA Reductase Inhibition on IGF-I-stimulated Proliferation and Differentiation of 3T3-L1 Preadipocytes—IGF-I (10 ng/ml (1.3 nM)) stimulated a 23-fold increase (p < 0.005) in [3H]thymidine uptake by 3T3-L1 preadipocytes in mid-log growth (Fig. 1A). From dose-response experiments,
it was determined that 5 μM insulin was required to achieve a similar fold induction to that of 10 ng/ml (1.3 nM) IGF-I (data not shown). The effects of both IGF-I and insulin could be significantly reduced by exposing cells to cerivastatin (3–300 nM) or staurosporine (Staur) 1 μM for 24 h before assessment of apoptosis by Hoechst staining and counting the number of cells with typically apoptotic nuclear morphology. Data are means ± S.D. of three separate experiments. B, cells were grown until 1 day post-confluent before treatment with cerivastatin (3–300 nM in fetal calf serum-containing medium) for 24 h. Cells were then maintained in differentiation medium plus cerivastatin for a further 2 days. Cell counts were used to assess the clonal expansion that precedes 3T3-L1 preadipocytes differentiation. Data are means ± S.D. from four independent experiments.

Since cerivastatin was the most potent drug tested, it was subsequently used to investigate the possibility of statin-induced apoptosis. Reduced proliferation was not due to drug-induced apoptosis since survival (determined by assessment of nuclear morphology) of cells treated with 3–300 nM cerivastatin for 24 h was similar to that of cells maintained in medium alone (Fig. 2A). Only by prolonged exposure (72 h) at a high dose of cerivastatin (300 nM) were we able to induce the level of apoptosis (29%) observed in response to a 24-h incubation with the known apoptotic agent staurosporine (Ref. 16 and data not shown).

Preadipocyte clonal expansion and subsequent differentiation is also dependent on a signal through the type 1 IGF receptor (17, 18). However, when cells were incubated with 3 or 30 nM cerivastatin, concentrations shown to significantly inhibit IGF-I-stimulated proliferation, clonal expansion, as assessed by the determination of cell number, was not significantly different from cells maintained in differentiation medium alone (Fig. 2B). Only exposure to 300 nM cerivastatin resulted in a significant (p < 0.001) reduction in cell number.

Effect of HMG-CoA Reductase Inhibition on [3H]2-Deoxy-glucose Uptake by 3T3-L1 Adipocytes—[3H]2-deoxy-glucose uptake by mature adipocytes was used to assess the effect of cerivastatin on the metabolic actions of IGF-I. Fig. 3A demonstrates that in contrast to its effect on the mitogenic actions of IGF-I, high doses of cerivastatin (300 nM) were necessary to evoke even a modest inhibition of IGF-I (100 ng/ml) stimulated [3H]2-deoxy-glucose uptake (28%; p < 0.005).

During adipocyte differentiation, insulin receptors are especially up-regulated, whereas IGF receptors are maintained at the level expressed by the preadipocyte; consequently, the mature cell responds to insulin as well as IGF-I, and we and others have shown that the two hormones are equipotent in promoting glucose uptake (19, 53). HMG-CoA reductase inhibition also abrogated the metabolic actions of insulin, but again, high doses (300 nM) were needed to attenuate significantly (32% reduction; p < 0.05) the stimulatory effect of insulin (Fig. 3B).

HMG-CoA Reductase Inhibitors Alter Processing of IGF and Insulin Receptors—HMG-CoA reductase inhibitors lead to the cellular depletion of mevalonate, and consequently, dolichyl phosphate, an essential cofactor in the process of N-linked protein glycosylation. It is therefore possible that mevalonate depletion might mediate its effect on IGF and insulin actions by disruption of receptor processing and thus receptor presentation at the cell surface. Initially, Western immunoblotting was used to assess the effect of cerivastatin on IGF and insulin receptor production. Fig. 4A shows that in preadipocytes incubated with cerivastatin (0.3–30 nM) for 24 h, there is a marked...
increase in the proportion of IGF-I proreceptors. Similarly, cerivastatin treatment of mature adipocytes led to an increase in the amount of insulin proreceptor, and in these cells, an alternate high molecular weight form of the insulin proreceptor was also observed (Fig. 4B). These results suggest that cerivastatin-treated cells are less efficient at processing the proreceptor to the mature α- and β-subunits. This should lead to decreased expression at the cell surface, and indeed, immunoprecipitation of lysates prepared from surface biotinylated NWT3b and 3T3-IR cells demonstrated reduced levels of IGF-I and insulin (respectively) receptor subunits as a result of treatment with cerivastatin (Fig. 5).

**HMG-CoA Reductase Inhibitors Affect Prenylation of Proteins Associated with IGF-I Signaling Pathways**—Since HMG-CoA reductase inhibition reduced the cell surface expression of receptors for both IGF-I and insulin, then altered receptor glycosylation alone cannot account for the differential affect of mevalonate depletion on the mitogenic and metabolic actions of IGF-I. We therefore investigated whether a reduction in farnesylation (FTI-277), we then investigated the relative importance of these post-translational modifications in transmission of IGF mitogenic and metabolic signals.

IGF-I-stimulated proliferation of 3T3-L1 preadipocytes was dose dependently decreased by all glycosylation inhibitors (0.25 μg/ml tunicamycin, p < 0.005, 10 μg/ml castanospermine, p < 0.01, 1 mM DMJ, p < 0.01), with tunicamycin, unsurprisingly, having the most severe effect (Fig. 7A). The farnesyl transferase inhibitor FTI-277 also inhibited IGF-I-mediated mitogenesis, suggesting that the severe effect of cerivastatin on IGF-I-stimulated cellular proliferation is due to a combination of disrupted receptor glycosylation and altered prenylation of intracellular signaling molecules. Insulin-stimulated glucose uptake was also inhibited by all glycosylation inhibitors (25 μg/ml tunicamycin, p < 0.01, 500 μg/ml castanospermine, p < 0.05, 20 mM DMJ, p < 0.05) with tunicamycin again having the most severe effect (Fig. 7B). FTI-277 had no effect on insulin-stimulated glucose uptake, confirming that the insulin metabolic pathway is sensitive to changes in receptor glycosylation but is not dependent on a prenylated signaling intermediate.

**DISCUSSION**

These studies clearly demonstrate that HMG-CoA reductase inhibitors are potent inhibitors of insulin- and IGF-mediated proliferation in 3T3-L1 cells. This high degree of potency is due to the combined disruption of prenylation pathways and glycosylation-dependent proreceptor cleavage of IGF receptors. In terminally differentiated adipocytes, the metabolic effects of insulin or IGF-I on glucose uptake are substantially less affected by HMG-CoA reductase inhibition; in these cells, prenylation pathways are of lesser importance with the major abnormality remaining impaired insulin and IGF receptor processing.
HMG-CoA reductase inhibitors are a group of drugs suggested to have significant pleiotropic effects (20–22). HMG-CoA reductase inhibitors decrease the proliferation of a range of cells (23–27) through the depletion of cellular mevalonate. Mevalonate depletion may lead to altered glycosylation of membrane proteins (25, 28–30) or prenylation of membrane-associated proteins (31–34). We show that in 3T3-L1 cells, it is the combined effect of these mechanisms that markedly abrogate mitogenic response of the cells to IGF-I, whereas their effect on metabolic signaling, only affected by one mechanism, is less markedly reduced.

IGF- and insulin-stimulated cell proliferation was significantly inhibited by all the HMG-CoA reductase inhibitors tested. Cerivastatin was the most potent, significantly inhibiting IGF-induced proliferation at 10 nM (p < 0.005), whereas pravastatin was the least potent, requiring 10 μM before achieving substantial inhibition of IGF-mediated cell proliferation. The order of potency, at inhibiting IGF-stimulated proliferation, correlates with their degree of hydrophobicity (ceriva > simva > flerva > atorva > prava), not their potency in inhibiting HMG-CoA reductase (atorva > ceriva > simva > flerva > prava) (35). Although hepatocytes take up statins by an active mechanism, fibroblasts do not (35). Thus in cell types without an active uptake mechanism, it appears that hydrophobicity confers the greatest effect on prenylation and glycosylation pathways.

The dramatic reduction in cell proliferation resulting from statin exposure occurs at concentration and exposure times far less than those necessary to induce apoptosis. Only after prolonged treatment with a high concentration of HMG-CoA reductase inhibitor were we able to demonstrate that IGF signaling had been disrupted sufficiently to induce apoptosis. Other studies have detected statin-induced apoptosis in a variety of cells (36–38). Similarly, these studies required either very high concentrations of statins over short periods (38) or prolonged exposure to lower concentrations (36, 37).

One mechanism by which HMG-CoA reductase inhibitors may alter signaling through the IGF and insulin pathways is through aberrant glycosylation of the IGF and insulin receptors. HMG-CoA reductase inhibition reduces formation of dolichyl phosphate, a carbohydrate donor during N-linked glycosylation of membrane-targeted proteins (10, 39). Due to their unusual requirement for correct N-linked glycosylation prior to the appropriate pro-receptor cleavage (11), the insulin and IGF receptor systems appear particularly prone to disruption by HMG-CoA reductase inhibitors (12, 30, 40–43). We have clearly shown that HMG-CoA reductase inhibition markedly alters both the processing and the cell surface expression of both the IGF and the insulin receptors in 3T3-L1 preadipocytes and adipocytes, respectively. This concurs with studies in melanoma and Ewing’s sarcoma cells in which an HMG-CoA reductase inhibitor or the glycosylation inhibitor tunicamycin down-regulated the number of IGF-Rs at the cell surface (30, 43). Similar changes in receptor processing were demonstrated by Hwang and Frost (12), in which glycosylation of insulin receptors was disrupted by depriving mature 3T3-L1 adipocytes of glucose. Under these conditions, increased amounts of lower molecular weight preproreceptors were formed that were subsequently degraded. We have shown that selective disruption of receptor glycosylation using specific inhibitors for the presence, length, and complexity of the carbohydrate moiety profoundly abrogates IGF and insulin receptor function. These findings are atypical. Most other membrane proteins appear less sensitive to changes in their glycosylation status (44). The
transport of lysosomal acid phosphatase was dependent on the presence but not the complexity of its N-linked oligosaccharides (45), whereas lipoprotein lipase requires carbohydrate presence for secretion and activity, but prevention of glycan processing with DMJ had no effect (46).

Mevalonate depletion also affects the isoprenylation of membrane-associated proteins, such as Ras. The depletion of farnesyl pyrophosphate with HMG-CoA reductase inhibitors leads to decreased signaling through Ras. Mitogenic but not metabolic signaling was inhibited by the farnesyl transferase inhibitor FTI-277, and inhibition of proliferation by cerivastatin was partially reversed by co-treatment with exogenous farnesyl pyrophosphate. Disturbed farnesylation is thus in part responsible for the inhibition of proliferation caused by cerivastatin, a process confirmed by reduced Ras association with isolated cell membranes in treated cells. Similar results have been obtained with lovastatin. In SK-MEL-2 cells, mevalonate depletion by lovastatin resulted in Ras prenylation being inhibited by 50% (47). Lovastatin also induced apoptosis in malignant mesothelioma cell lines, which was partially reversed by farnesol refeeding (48). In these cells, there was a relocalization of Ras from the membrane to the cytosol. In human glioma cell lines, lovastatin decreased cellular proliferation by 80% due to decreased signaling through Ras (31). Prior exposure of cells to insulin (and possibly IGF-I) may potentiate the subsequent actions of other mitogens (49). Drzazin and co-workers (50–52) determined that insulin causes the phosphorylation and activation of farnesyl transferase, which in turn leads to a greater proportion of farnesylated and membrane-associated Ras. By blunting the effect of insulin, HMG-CoA reductase inhibitors may abrogate this effect on two levels, by decreasing the insulin activation of farnesyl transferase and by depleting the cell of farnesyl pyrophosphate.

In summary, mevalonate depletion affects IGF and insulin signaling via at least two distinct but related and synergistic mechanisms: reduced prenylation of signaling intermediates such as Ras and disruption of insulin and IGF proreceptor processing. IGF-induced mitogenic processes in rapidly proliferating cells are more potentely affected than insulin-mediated metabolic processes in terminally differentiated cells. Although the effects on receptor processing in most terminally differentiated cells may have little influence, those cells with active uptake mechanisms for statins may be substantially affected. In such cell types, insulin and IGF resistance might be anticipated.

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