Tyrosine kinase-dependent modulation of 3-hydroxy-3-methylglutaryl-CoA reductase in human breast adenocarcinoma SKBR-3 cells

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3-Hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase is the major rate-limiting enzyme in sterol and non-sterol isoprenoid synthesis. Isoprenoids are involved in the mechanisms of cell proliferation and transformation leading notably to crucial post-translational maturation of small G-proteins of the Ras superfamily. HMG-CoA reductase is among the most highly regulated enzymes. It is controlled by several feedback regulation mechanisms induced by sterol and non-sterol metabolites. The present results show that tyrosine kinase activity is also involved in the regulation of HMG-CoA reductase activity in the human breast cancer cell line SKBR-3. Incubation of SKBR-3 cells with the tyrosine kinase inhibitor, herbimycin A, induces a concentration-dependent reduction of HMG-CoA reductase activity with an IC_{50} of 80 nM. The inhibition of HMG-CoA reductase activity by herbimycin A is also time-dependent. A similar effect of

herbimycin A was obtained on the steady-state level of the HMG-CoA reductase protein. The effect of herbimycin A is probably specific as it abolished the stimulation of reductase activity by epidermal growth factor. To elucidate the molecular basis of the inhibition of HMG-CoA reductase activity and protein level by herbimycin A, we performed experiments to study the metabolic turnover of this enzyme using [³⁵S]methionine and [³⁵S]cysteine. Herbimycin A (1 μ M) did not have any significant effect on the rate of HMG-CoA reductase protein degradation but did affect its rate of synthesis and mRNA levels. The decrease in protein synthesis rate correlates with the lower reductase protein level but is more pronounced than the decrease in mRNA levels. Taken together, the results reveal a novel pathway of regulation of HMG-CoA reductase expression and activity by cellular tyrosine kinase activities.

INTRODUCTION

3-Hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase) is the major rate-limiting enzyme of the mevalonate pathway involved in synthesis of both sterol and non-sterol isoprenoids (including ubiquinone, dolichol, isopentenyladenine and prenyl groups), which are compounds required for cell survival and proliferation. HMG-CoA reductase is a glycoprotein of 97 kDa anchored mainly in the endoplasmic reticulum membrane and is one of the most highly regulated enzymes. Its regulation occurs by several feedback mechanisms induced by sterol and non-sterol metabolites at transcriptional and post-transcriptional levels [1]. Repression of transcription of the HMG-CoA reductase gene by sterols is dependent on a sterol-regulatory region in the 5'-flanking region of the gene [2]. Translational control of HMG-CoA reductase by non-sterol metabolites was initially described by Peffley and Sinensky [3] and confirmed by Nakanashi *et al.* [4]. Post-translational regulation of reductase occurs through a degradative process, which is accelerated by both sterol and non-sterol products. Hence the combination of transcriptional and post-transcriptional controls can regulate the amount of reductase protein over a several hundred-fold range in animal cells [4]. Moreover the specific activity of the enzyme is controlled by phosphorylation *via* an AMP-activated protein kinase resulting in decreased activity and increased sensitivity to degradation [5,6]. Recently cAMP has been implicated in the regulation of HMG-CoA reductase [7]. Thus in FRTL-5 cells, HMG-CoA reductase is transcriptionally induced by thyrotropin *via* a cAMP-

responsive element in the 5'-flanking region of the reductase promoter [8].

Extensive studies have demonstrated that certain mevalonate-derived products are involved in the mechanisms controlling DNA synthesis and cell proliferation [9–13]. Inhibition of HMG-CoA reductase activity by statins or oxysterols leads to a decrease in cell proliferation. Tumour cells in contrast exhibit an increase in HMG-CoA reductase activity, protein and/or mRNA content [14–16]. A positive correlation between cell stimulation with growth factors and increase in HMG-CoA reductase activity has been established. Hence the addition of platelet-derived growth factor (PDGF) to quiescent Swiss 3T3 cells [17] or human fibroblasts increased the activity of HMG-CoA reductase several-fold [18,19]. Similar results have been obtained in other types of cell activated by growth factors or serum [20,21].

It has previously been shown in our laboratory that HMG-CoA reductase displays a higher activity towards non-sterol products in the adenocarcinoma cell line A549 than in normal cells [22] and that the reductase activity could be involved in cell transformation [23]. Tyrosine kinase activities are known to be critical in the regulation of normal cellular growth, and activation of these kinases is frequently reported to be associated with malignant transformation [24]. The erbB receptor family, notably epidermal growth factor (EGF) receptor and p185^{erbB-2}, has often been implicated in human neoplasia. Overexpression of these receptor tyrosine kinases, in particular, has been found in a significant percentage of breast cancers and has been implicated in poor prognosis [25–28]. Likewise the Src family of non-

Abbreviations used: EGF, epidermal growth factor; FCS, fetal calf serum; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; PDGF, platelet-derived growth factor.

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receptor tyrosine kinases is critically involved in the proliferation of breast cancer cells [29]. Tyrosine kinase inhibitors with broad specificity, such as herbimycin A and genistein, have recently been shown to inhibit the proliferation of SKBR-3 and MCF-7 cells, two breast cancer cell lines [30].

To investigate the possible relationship between tyrosine kinase activity and isoprenoid metabolism, we studied the regulation of HMG-CoA reductase by tyrosine kinase activity. We chose to analyse the human breast adenocarcinoma SKBR-3 cells because they express a high level of p185^{erbB-2} [31]. Using herbimycin A and EGF we show for the first time that modulation of tyrosine kinase activity leads to regulation of HMG-CoA reductase.

MATERIALS AND METHODS

Cell culture

The human breast adenocarcinoma SKBR-3 cells (ATCC-HTB 30) were routinely grown at 37 °C in RPMI 1640 medium supplemented with 5 % fetal calf serum (FCS) (Gibco-BRL) under standard conditions of humidity and CO₂ atmosphere.

Assay of HMG-CoA reductase activity

SKBR-3 cells (4×10^5) seeded in 100 mm dishes were grown in RPMI 1640 medium supplemented with 5 % FCS (growth medium) for 4 days. They were then treated for the indicated time with increasing concentrations of herbimycin A (Calbiochem) or vehicle (DMSO). They were washed with ice-cold PBS and kept on ice before being scraped and lysed for 30 min at 4 °C in the presence of lysis buffer (50 mM potassium phosphate, pH 7.4, 5 mM EDTA, 150 mM NaCl, 1 % Triton X-100, 10 µg/ml leupeptin, 10 µg/ml pepstatin and 1 mM PMSF). Cell lysates were cleared at 4 °C by centrifugation at 12000 *g* for 15 min. The supernatants were collected for determination of total HMG-CoA reductase activity. Approx. 50–150 µg of protein in 25 µl of lysis buffer was preincubated at 37 °C for 30 min. Reactions were started by the addition of 27 µl of incubation buffer containing 230 mM potassium phosphate, pH 7.5, 10 mM dithiothreitol, 23 mM NaCl, 0.35 unit of glucose-6-phosphate dehydrogenase (Sigma), 40 mM glucose 6-phosphate, 5 mM NADP⁺, 0.15 mM HMG-CoA and [3-¹⁴C]HMG-CoA (58 mCi/mmol). After an incubation of 45 min at 37 °C, the reactions were stopped by the addition of 10 µl of 6 M HCl, and the samples were further incubated for 30 min at 37 °C. The [¹⁴C]mevalonate formed was separated from HMG-CoA on a 5 cm column of Bio-Rex® resin (100–200 mesh; Bio-Rad).

The protein concentration of the cell lysates was determined by the method of Bradford [32] using the Bio-Rad protein dye and BSA as a standard.

Cell labelling

SKBR-3 cells were labelled with ³⁵S in methionine- and cysteine-free RPMI 1640 (Sigma) containing 5 % (v/v) dialysed FCS. Before labelling, 4×10^5 cells grown in 100 mm dishes for 4 days were washed twice with PBS and incubated for 30 min in 3 ml of methionine- and cysteine/cystine-free medium. The cells were then labelled in the same medium in the presence of 100–200 µCi of [³⁵S]methionine and [³⁵S]cysteine (Tran³⁵S-label; 1142 Ci/mmol; ICN Radiochemicals) before being washed twice with ice-cold PBS and chased for various periods of time in complete medium. At the end of the treatment, the cells were lysed in lysis buffer and the HMG-CoA reductase immunoprecipitated.

Immunoprecipitation and Western-blot analysis of HMG-CoA reductase

Cellular protein (300 µg) was incubated overnight at 4 °C in the presence of rabbit polyclonal anti-(HMG-CoA reductase) antibody (a gift from Dr. Germershausen, Merck Research Laboratories, Rahway, NJ, U.S.A.). Immune complexes were collected after 90 min of incubation at 4 °C with 30 µl of Protein A-Sepharose (Sigma). Beads were washed twice with lysis buffer and twice with PBS. The immune complexes were released by a 5 min incubation at room temperature with 15 µl of 'elution buffer' (Bio-Rad). Immunoprecipitates were resolved by SDS/PAGE (8 % gels). Gels were fixed with 10 % acetic acid/50 % methanol, dried and exposed to Amersham MP film at –80 °C or electroblotted on to nitrocellulose membrane (Bio-Rad). After being blocked for 45 min at room temperature with 5 % non-fat dry milk in TBST (25 mM Tris/HCl, pH 7.8, 140 mM NaCl, 0.05 % Tween 20), the membranes were incubated for 3 h with the monoclonal anti-(HMG-CoA reductase) antibody (mouse IgG ATCC A9) in TBST. The membranes were then washed extensively with TBST and incubated for 1 h with horseradish peroxidase-coupled antibodies (directed against mouse IgG) (Bio-Rad). Detection was performed with the enhanced chemiluminescence system (Amersham).

Northern-blot analysis of HMG-CoA reductase mRNA

SKBR-3 cells were seeded at a density of 4×10^5 /100 mm dish and grown for 4 days in the growth medium. After treatment, cells were washed twice with PBS, and the total RNA from SKBR-3 cells was isolated with TRIzol Reagent (Gibco) by a modification of the guanidinium/phenol/chloroform method [33]. Total RNA (20 µg) was denatured in solution containing 50 % deionized formamide, 6.6 % formaldehyde and 1 × Mops (0.02 M Mops, 5 mM sodium acetate, pH 7, 1 mM disodium EDTA, pH 8) for 10 min at 65 °C. After being refreshed on ice, they were electrophoresed on 1.2 % agarose gel in the presence of formaldehyde (6.6 %), transferred to a Hybond-N membrane (Amersham) and fixed for 2 h at 80 °C. Membranes were then prehybridized at 42 °C for 2 h. Hybridization was carried out with a human DNA probe for HMG-CoA reductase and a β-actin probe (as a control) which were labelled with [α-³²P]dCTP using a nick translation kit (Boehringer). After 24 h of incubation, the membranes were washed three times for 15 min in a solution consisting of 2 × SSC (1 × SSC = 0.15 M NaCl/15 mM sodium citrate) and 0.5 % SDS at 37 °C and twice for 1 h in 0.2 × SSC/0.1 % SDS at 55 °C. Autoradiograms of hybridized membranes were obtained using hyperfilm MP (Amersham) exposed at –80 °C. HMG-CoA reductase mRNA levels were quantified by densitometry scanning of autoradiographs using a Nikon video camera linked to a personal computer and the Optimas program and normalized to the β-actin mRNA signal.

RESULTS

Inhibition of HMG-CoA reductase activity in SKBR-3 cells by herbimycin A

The ability of herbimycin A to modulate HMG-CoA reductase activity in SKBR-3 cells was examined. After a 4 h incubation with herbimycin A, HMG-CoA reductase activity was significantly reduced in a dose-dependent manner (Figure 1A). Herbimycin A inhibited HMG-CoA reductase activity with an IC₅₀ of 80 nM. Near-maximum of inhibition of HMG-CoA reductase activity (70 %) was obtained at a herbimycin A concentration of 1 µM. The time-course effect of 1 µM herbimycin A on HMG-CoA reductase activity is presented in Figure 1(B).

Figure 1 Effect of herbimycin A on HMG-CoA reductase activity

SKBR-3 cells 4×10^5 were seeded into 100 mm dishes and grown in RPMI 1640 medium supplemented with 5% FCS for 4 days. They were then treated for 4 h with increasing herbimycin A concentrations (A) or with $1 \mu\text{M}$ herbimycin A for different times (B). Cells were then solubilized and the lysates used for determination of total HMG-CoA reductase activity. Results are expressed as percentage of control HMG-CoA reductase activity. Each value represents the mean $\pm$ S.E.M. from four to ten independent experiments.

Figure 2 EGF-induced stimulation of HMG-CoA reductase activity

SKBR-3 cells were grown in RPMI 1640 medium with 5% FCS. After 4 days of growth, the medium was renewed and 55 ng/ml EGF was added. After different times of exposure of EGF, the cells were lysed and HMG-CoA reductase activity was determined. Each value represents the mean $\pm$ S.E.M. from two to three independent experiments performed in duplicate.

Inhibition of HMG-CoA reductase activity by herbimycin A was already observed after 2 h incubation and attained a maximum after 8 h. No further decrease in activity was observed after 24 h (results not shown).

As cell morphology was altered after 8 h of treatment with the inhibitor (suggesting a cytotoxic effect), we chose to use a 4 h treatment in the following experiments.

Stimulation of HMG-CoA reductase activity in SKBR-3 cells by EGF

A ligand for tyrosine kinase receptor p185^{erbB-2} has not yet been identified. However, treatment of SKBR-3 cells with EGF produces an increase in erbB-2 phosphorylation on tyrosine residues which can be explained by heterodimerization of p185^{erbB-2} with EGF receptor [34]. Thus, to verify that HMG-CoA reductase could be under the control of tyrosine kinase activity, we examined the effect of EGF on the reductase activity. As shown in Figure 2, exposure of SKBR-3 cells to 55 ng/ml

Table 1 Effects of EGF on HMG-CoA reductase activity in the presence of herbimycin A

SKBR-3 cells were pretreated for 15 min with or without $1 \mu\text{M}$ herbimycin A, and then 55 ng/ml EGF was added or not for 4 h. Cells were solubilized and the lysates were used to determine total HMG-CoA reductase activity. Results are expressed as a percentage of the control activity. Each value represents the mean $\pm$ S.E.M. from three independent experiments.

Herbimycin A	HMG-CoA reductase activity (%)	
	Without EGF	With EGF
None	100	204.5 $\pm$ 17.5
$1 \mu\text{M}$	28.2 $\pm$ 3.6	91.0 $\pm$ 7.4

EGF for various times led to a significant increase in HMG-CoA reductase activity. The stimulation of HMG-CoA reductase activity reached a plateau after 4 h at which point the reductase activity was increased twofold.

To see whether the effect of herbimycin A on HMG-CoA reductase activity was specifically the result of tyrosine kinase inhibition, we investigated the capacity of herbimycin A to inhibit EGF-induced stimulation of reductase activity. SKBR-3 cells were treated or not with 55 ng/ml EGF in combination with $1 \mu\text{M}$ herbimycin A for 4 h. As expected, herbimycin A alone induced almost complete inhibition of HMG-CoA reductase activity, and the addition of EGF induced a twofold increase in HMG-CoA reductase activity (Table 1). This stimulation of reductase activity by EGF was abolished when $1 \mu\text{M}$ herbimycin A was added concurrently with EGF. However, the effect of herbimycin A in combination with EGF on HMG-CoA reductase activity was not as high as when added alone (Table 1). Similar results have previously been obtained for the phosphorylation of Shc in SKBR-3 cells [30]. Experiments were therefore undertaken to study the effect of tyrosine kinase activity on the different steps of HMG-CoA reductase biosynthesis.

Dose-dependence and time-course effect of herbimycin A on HMG-CoA reductase protein level

We first investigated whether the herbimycin A-induced reduction of HMG-CoA reductase activity correlated with modification of

Figure 3 Effect of herbimycin A on HMG-CoA reductase protein levels

SKBR-3 cells in exponential growth phase were treated with 1 μ M herbimycin A for various times (A and B) or for 4 h with increasing concentrations of herbimycin A (C). HMG-CoA reductase was immunoprecipitated and analysed by Western blot as described in the Materials and methods section. Densitometric analysis of the area corresponding to the 97 kDa band was performed and the densitometry units obtained are shown in (B) and (C).

the reductase protein level in SKBR-3 cells. The reductase was immunoprecipitated using an antibody specific to the catalytic domain of the enzyme and then subjected to Western-blot analysis. As shown in Figure 3(A), herbimycin A induced a time-dependent depletion of reductase protein in SKBR-3 cells. Densitometry analysis of the area corresponding to the 97 kDa band on the autoradiograph revealed that exposure of cells to 1 μ M herbimycin A for 4 h produced rapid and almost complete depletion of this protein (Figure 3B). Since the decrease in reductase protein content occurred to the same extent as the inhibition of reductase activity, inhibition caused by alkylation of the reductase by herbimycin A could be excluded. When cells were incubated with herbimycin A for 4 h, the reductase protein

level was reduced in a dose-dependent manner (Figure 3C) as previously observed for reductase activity (Figure 1A).

Effect of herbimycin A on rate of HMG-CoA reductase protein degradation and synthesis

The post-transcriptional control of HMG-CoA reductase, which occurs through a degradation process, is known to constitute a major step in HMG-CoA reductase regulation [1]. Thus, to elucidate the molecular basis of the reduction in HMG-CoA reductase level by herbimycin A, we examined the enzyme half-life in a pulse-chase experiment with [35 S]methionine and [35 S]cysteine. After labelling for 5 h, the chase was performed in the presence or absence of 1 μ M herbimycin A. The kinetics of disappearance of labelled HMG-CoA reductase are shown in Figure 4(A). The addition of herbimycin A did not lead to a significant acceleration of reductase protein degradation. The calculated half-lives of HMG-CoA reductase protein were 3.7 ± 0.5 h and 3.4 ± 0.2 h in the absence and presence of herbimycin A respectively. The difference between the two regression lines (which have r values of respectively 0.9533 and 0.9963) was not significant within a confidence interval of 95%.

Thus the decrease in HMG-CoA reductase protein levels may be the consequence of reduced protein synthesis. Indeed analysis of the 1 μ M herbimycin A effect on the rate of HMG-CoA reductase synthesis after a pulse experiment with [35 S]methionine and [35 S]cysteine showed that HMG-CoA reductase protein synthesis was already decreased after 1 h. A fourfold inhibition of reductase synthesis was reached after 4 h (Figure 4B).

Decrease in HMG-CoA reductase mRNA levels induced by herbimycin A

Further experiments were performed to determine whether the decrease in HMG-CoA reductase protein synthesis was the result of a reduction in HMG-CoA reductase mRNA content. The mRNA content in treated SKBR-3 cells was investigated by Northern-blot analysis. As indicated in Figure 5, treatment of SKBR-3 cells with 1 μ M herbimycin A resulted in a decrease in HMG-CoA reductase mRNA. Within 4 and 8 h of incubation, HMG-CoA reductase mRNA levels were decreased by 60.0 ± 2.7 and 34.5 ± 5.6 % of the control respectively. It thus appears that modulation of HMG-CoA reductase mRNA was slower than that of the protein.

DISCUSSION

HMG-CoA reductase is known to be a key enzyme in the mevalonate pathway. It has been shown to be positively associated with DNA synthesis and proliferation of mammalian cells [35,36]. Abnormal reductase expression seems to be a characteristic of tumour cells and is associated with similar disorders in low-density lipoprotein receptor expression [37]. A part of low-density lipoprotein receptor expression is regulated by the tyrosine kinase pathway [38], and this proportion is higher when the cells are actively dividing [39]. Regulation of HMG-CoA reductase by the tyrosine kinase activity of PDGF receptor has been recently suggested [19]. The question then arises as to whether HMG-CoA reductase expression can also be modulated by tyrosine kinase activity. This is why we chose to study HMG-CoA reductase regulation in a mammary tumour cell line (SKBR-3) that overexpresses a tyrosine kinase receptor (p185^{erbB-2}) that is partially tyrosine-autophosphorylated in a constitutive manner.

Herbimycin A is a general tyrosine kinase inhibitor that has been shown to irreversibly block intracellular tyrosine kinases

Figure 4 Effect of herbimycin A on rate of degradation and synthesis of HMG-CoA reductase protein

Cells were allowed to grow in 5% FCS/RPMI 1640 medium for 4 days. They were then pulse-labelled as described in the Materials and methods section. (A) At the end of the 5 h of pulsing, cells were collected ($t = 0$) or chased for 0.5–8 h in the complete medium supplemented (●) or not (○) with 1 μ M herbimycin A. At the indicated times, the cells were collected, lysed and the 35 S-labelled HMG-CoA reductase was immunoprecipitated and subjected to SDS/PAGE (8% gel). The bands corresponding to HMG-CoA reductase protein were quantified by densitometric scanning. The ratio of the residual amount (E) of HMG-CoA reductase determined at the indicated times to the initial amount of HMG-CoA reductase (E_0) determined at time 0 is plotted. The graph is representative of three experiments, and linear regressions were calculated with intervals of 95% confidence. (B) After the indicated labelling time, 35 S-labelled HMG-CoA reductase from cells exposed (●) or not (○) to 1 μ M herbimycin A was immunoprecipitated and subjected to electrophoresis. The densitometric units determined from the autoradiographs are presented. The graph is representative of three experiments.

Figure 5 Decrease in HMG-CoA reductase mRNA content caused by herbimycin A

Cells were grown in 5% FCS/RPMI 1640 medium for 4 days. They were then incubated in the presence or absence of 1 μ M herbimycin A in complete medium at 37 °C. At the indicated times, total RNA was isolated from four pooled 100 mm dishes and analysed by Northern blot as described in the Materials and methods section (A). The amount of HMG-CoA reductase mRNA was quantified by densitometric scanning of autoradiographs after normalization to the β -actin mRNA signal (B).

Src and Bcr-Abl, EGF receptor and ErbB-2 by alkylation of thiol groups [24]. At a concentration of 0.35 μ M, it can reduce the tyrosine phosphorylation of p185^{ErbB-2} in SKBR-3 cells by 80% after 2 h, and alkylation of p185^{ErbB-2} is followed by its depletion [40]. The inhibition of HMG-CoA reductase activity by herbimycin A is shown here to be dose- and time-dependent. It was accompanied by a similar decrease in the level of the reductase protein, which was itself shown to be related to a lower rate of synthesis. In contrast, protein degradation was unaffected by herbimycin A. The lower rate of protein synthesis was accompanied by a decrease in reductase mRNA levels. Tyrosine kinase involvement is supported by the twofold increase in HMG-CoA reductase activity when the cells were treated with EGF. The specificity of herbimycin A is indicated by the absence of stimulation by EGF when the cells were treated with herbimycin A before the addition of EGF.

The decrease in HMG-CoA reductase protein level caused by herbimycin was due exclusively to inhibition of reductase protein synthesis. Figure 3(B) shows a 75% inhibition of the steady-state protein level (expressed as percentage of control) at 4 h, and Figure 4(B) shows a similar 75% inhibition of reductase synthesis at 4 h. However, the inhibition of reductase mRNA only attained a value of 40%, which could not account for the 75% decrease in protein synthesis rate. The mechanism by which the rate of reductase synthesis was decreased must therefore involve not only lower mRNA levels but also other post-transcriptional events.

Apart from the classical retro-inhibition mechanisms of HMG-CoA reductase activity by sterol and non-sterol compounds, certain other regulatory mechanisms have been described. (1) Thyroid hormones are able to decrease mRNA levels of HMG-CoA reductase in rat liver [41]. (2) A cAMP-responsive element has been characterized in the reductase promoter of rat thyroid cells [8], depletion of cAMP in these cells impairing phosphorylation of the cAMP-responsive element-binding protein by protein kinase A and occurring concomitantly with an increase in protein kinase C activity [42]. (3) Tyrosine kinase activity may be

involved, as insulin can modulate reductase mRNA levels in diabetic rats [43] and PDGF stimulates reductase activity and expression of insulin-like growth factor I receptors in human fibroblasts [44]. Our results are the first to demonstrate the involvement of tyrosine kinase activity in the regulation of HMG-CoA reductase. This new regulation mechanism may account for previous observations that insulin and PDGF can stimulate reductase expression.

The modulation of HMG-CoA reductase by tyrosine kinase can be related to the effects of the activities of these two enzymes on cell proliferation and differentiation. Over the past few years, the importance of the role of HMG-CoA reductase in cell growth and differentiation has been increasingly recognized. Competitive inhibitors of HMG-CoA reductase induce cell-cycle arrest in G₁ phase [45]. HMG-CoA reductase activity has also been shown to be related to cell transformation [23] and to mediate the growth-inhibitory effects of retinoic acid in neuroblastoma cells [16]. On the other hand, proliferation of the human breast cancer cell line SKBR-3 is inhibited by the tyrosine kinase inhibitors herbimycin A and genistein. This effect has been shown to be accompanied by decreased Ras activation and mitogen-activated protein kinase activity [30].

This importance of HMG-CoA reductase in cell proliferation and differentiation can be related to the essential roles of prenylated proteins in these processes. Dividing cells require more cholesterol for synthesizing membranes but have an overall need for isoprenoid compounds that are essential for various signalling pathways: (1) activation of 'switch proteins', such as Ras and Rho, that can activate mitogen-activated protein kinases and induce cytoskeletal modifications; (2) N-linked glycosylation which is necessary for translocation of growth factor receptors to the cell surface [44].

It is worth mentioning that the regulation of HMG-CoA reductase by tyrosine kinase observed here occurs in the presence of cholesterol lipoproteins of FCS and therefore appears to be independent of cholesterol requirements. The stimulation of HMG-CoA reductase expression by tyrosine kinase activity may therefore constitute a regulatory pathway that is physiologically essential in actively dividing cells and that can contribute to tumoural cell growth.

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