The HMG-CoA reductase inhibitor simvastatin overcomes cell adhesion–mediated drug resistance in multiple myeloma by geranylgeranylation of Rho protein and activation of Rho kinase

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Primary drug resistance is a major problem in multiple myeloma, an incurable disease of the bone marrow. Cell adhesion-mediated drug resistance (CAM-DR) causes strong primary resistance. By coculturing multiple myeloma cells with bone marrow stromal cells (BMSCs), we observed a CAM-DR of about 50% to melphalan, treosulfan, doxorubicin, dexamethasone, and bortezomib, which was not reversed by secreted soluble factors. Targeting the adhesion molecules lymphocyte function–associated antigen 1 (LFA-1) and very late antigen 4 (VLA-4) by monoclonal antibodies or by the LFA-1 inhibitor LFA703 reduced CAM-DR significantly. Only statins such as simvastatin and lovastatin, however, were able to completely restore chemosensitivity. All these effects were not mediated by de-adhesion or reduced secretion of interleukin 6. Targeting geranylgeranyl transferase (GGTase) and Rho kinase by specific inhibitors (GGT-298 and Y-27632), but not inhibition of farnesytransferase (FTase) by FTI-277, showed similar reduction of CAM-DR. Addition of geranylgeranyl pyrophosphate (GG-PP), but not of farnesyl pyrophosphate (F-PP), was able to inhibit simvastatin-induced CAM-DR reversal. Our data suggest that the 3-hydroxy-3-methylglutaryl-coenzyme-A (HMG-CoA)/GG-PP/Rho/Rho-kinase pathway mediates CAM-DR and that targeting this pathway may improve the efficacy of antimyeloma therapies by reduction of CAM-DR. (Blood. 2004;104:1825-1832)

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Introduction

In multiple myeloma, malignant plasma cells are localized to the bone marrow in close association with stromal cells and are rarely found in other locations. Almost all symptoms and complications of this disease are due to this bone marrow infiltration. In the bone marrow, the myeloma cells and stromal cells secrete cytokines and interact through adhesion molecules, activating the stromal cells that further support the growth and survival of the myeloma cells. Furthermore, it has been shown recently that myeloma cells in the bone marrow microenvironment are much less sensitive to chemotherapeutic drugs. This mechanism of primary drug resistance has been termed cell adhesion–mediated drug resistance (CAM-DR). It was shown that CAM-DR is not associated with reduced drug accumulation and that adhesion molecules such as very late antigen 4 (VLA-4) are overexpressed in resistant myeloma cell lines. Despite recent advances in the therapy of multiple myeloma using dose-intensified regimens and new molecular-targeted compounds such as IMiDs immunomodulatory drugs [CC-5013] or proteasome inhibitors (bortezomib), the disease still remains incurable. Patients eventually become resistant to the most common and most potent drugs has not yet been performed. Furthermore, no drugs are currently available to circumvent CAM-DR. Therefore, we performed this comprehensive study of the adhesion-mediated multidrug resistance in multiple myeloma and assessed possible targets for the circumvention of CAM-DR.

Materials and methods

Cells

NCI-H929, U266, RPMI-8226, OPM-2, and HS-5 cell lines were obtained from the American Type Culture Collection (Rockville, MD), grown in RPMI 1640 medium (Boehringer, Ingelheim, Germany) containing 20% heat-inactivated fetal calf serum (FCS; Boehringer) in a humidified atmosphere (37.5°C, 5% CO2), and seeded at a concentration of 1 x 105 cells/mL. After informed consent was obtained from patients, mononuclear cells from bone marrow aspirates were grown in plastic flasks to a confluent, adherent monolayer as described. The ethics committee of the University of Munich approved the study.

Reagents

Simvastatin and dexamethasone were purchased from Sigma-Aldrich (Seelze, Germany), melphalan from GlaxoSmithKline (Munich, Germany), treosulfan from Medac (Wedel, Germany), doxorubicin from Pharmacia and activation of Rho protein...
chemotherapeutic agents were added to the culture. After 48 hours, cell layer, HS-5, or human bone marrow stromal cells (hBMSCs), were from Sigma-Aldrich (Steinheim, Germany); and FITC-277, GGTTI-298, and Y-27632 were from Calbiochem (Darmstadt, Germany). LFA703 was kindly provided by Novartis (Basel, Switzerland). The 3-μm cell culture inserts and fibronectin (FN)-coated well plates were obtained from BD Falcon (Heidelberg, Germany). CD106, CD11a, and CD138 were purchased from Pharmingen (BD Biosciences, Heidelberg, Germany); CD38-fluorescein isothiocyanate (FITC), CD49d-FITC, and CD54-FITC were from Immunotech (Beckman Coulter, Krefeld, Germany) within 30 minutes.

Surface expression of antigens
Cells were stained by the manufacturer’s recommendations as described7 and expression was determined by flow cytometry.

Interleukin 6
Levels of interleukin 6 (IL-6) were measured by a commercially available enzyme-linked immunosorbent assay (ELISA; Medgenix, Ratingen, Germany).

De-adhesion assay
For quantification of the cells in suspension, a WST-1 viability assay protocol was used as recommended by the manufacturer (Roche, Penzberg, Germany). Supernatant with the cells in suspension was transferred to new wells of a microtiter plate and absorbance at 440 nm was measured using a microplate ELISA reader to detect metabolically intact cells (reference wavelength, 680 nm).

Statistics
Mean values with SDs from representative experiments are shown in the figures. The Kruskal-Wallis one-way analysis of variance on ranks was used to determine the statistical significance of treatment results. The pairwise multiple comparison procedure was performed according to the Dunn method. P less than .05 was considered statistically significant.

Results
Direct cell-cell interaction between multiple myeloma cells and BMSCs causes strong de novo multidrug resistance
The bone marrow microenvironment consists mainly of BMSCs and extracellular matrix proteins. In preliminary experiments we grew NCI-H929 and OPM-2 multiple myeloma cells in the presence and absence of FN. Cells were harvested and apoptosis was determined by aV and PI staining by flow cytometry. Figure 1A shows a trend toward reduction of cell death by adhesion to FN, which does not meet statistical significance in our setting. Furthermore, myeloma cells were grown on a monolayer of HS-5 BMSCs for ligation of alternative adhesion molecules. Staining with CD38-FITC or CD138-FITC antibodies identified myeloma cells and apoptosis/cell death was determined with PI by flow cytometry. Figure 1B demonstrates strong reduction of melphalan-induced cell death of about 50% in all 4 tested cell lines. U266 cells are already adherent to plastic and show reduced chemoresistance upfront, which is further reduced by coinubcation with HS-5 cells. Although cell death was the clinically most important end point of our study, we were interested in whether early apoptosis can also be diminished by cell adherence. With 5 μM instead of 30 μM melphalan, NCI myeloma cells did not become PI positive after 48 hours but showed breakdown of plasma membrane asymmetry as determined by binding of aV, demonstrating that adhesion to BMSCs prevents myeloma cells from early apoptosis (Figure 1C). To investigate whether this is a drug-specific effect of melphalan, we repeated the experiment using a variety of potent chemotherapeutic drugs such as treosulfan,6 doxorubicin, and dexamethasone (Figure 1D). Strong and statistically significant CAM-DR to all tested compounds could be detected. Cell death was reduced by 57%, 63%, 42%, 68%, and 73% as indicated in Figure 1D. Comparable strong drug resistance was seen using the other 3 myeloma cell lines (data not shown). Interestingly, adherent myeloma cells also show strong primary resistance to the proteasome inhibitor PS-341, which very recently has been demonstrated to be capable of overcoming FN adherence-mediated drug resistancea and which induces a good clinical response rate in 35% of patients with refractory myeloma.9 Similarly, apoptosis caused by the proteasome inhibitor MG132 was reduced by 60% following adherence to HS-5 cells (data not shown). In conclusion, we observed strong primary MDR in adherent myeloma cells. Because HS-5 is an immortalized, rapidly proliferating cell line, primary BMSC cultures from 3 consecutive patients were used for further coculture experiments. Figure 1E demonstrates that the extent of CAM-DR induced by primary BMSCs is comparable to CAM-DR seen with HS-5 cells, suggesting that HS-5 cells provide a good and simple in vitro model. Similar, statistically significant results were obtained using different concentrations of PS-341 with 4 additional patient samples (data not shown). A time-course experiment (Figure 1F) revealed that rescue from apoptosis/cell death is strongly dependent on the presence of BMSCs over the whole incubation period. Cytokines secreted by myeloma or stromal cells on adherence, such as IL-6 or vascular endothelial growth factor (VEGF), contribute to increased survival, proliferation, and drug resistance.10,11 To address this issue we used cell-culture inserts, which allow free exchange of soluble factors but completely abrogate direct cell-cell interaction. Multiple myeloma cells were grown in the upper chamber while an HS-5 monolayer was just below the membrane of the insert (transwell). Inhibition of cell-cell contact completely abolished drug resistance (Figure 1G). Others have shown that cytokines released by cancer cell-stromal cells interaction can protect cells from drug-induced cell death, suggesting that direct contact may not be absolutely necessary to protect the malignant cell. Therefore, transfer experiments with conditioned media were performed to determine the effects of constitutively (HS-5 medium) and adhesion-mediated (HS-5/NCI medium) secretion of soluble factors on CAM-DR. No significant influence of soluble factors on CAM-DR could be detected in our model (Figure 1H). These data support the observation that direct interaction is essential for CAM-DR. The most important adhesion molecules that mediate contact between myeloma cells and stromal cells are LFA-1 and VLA-4, which are up-regulated on treatment with cytotoxic agents.5 Furthermore, the corresponding ligands on HS-5 cells, vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1), are up-regulated even after 48 hours, suggesting increase of CAM-DR after chemotherapy (Figure 1I).
Targeting adhesion molecules may overcome CAM-DR

These data raised the question whether targeting of adhesion-mediated resistance could improve the outcome of standard chemotherapy regimens. The simultaneous addition of blocking anti–LFA-1 and anti–VLA-4 antibodies to melphalan revealed strong reduction of CAM-DR (Figure 2A), whereas the anti–VLA-4 or anti–LFA-1 antibodies alone had no effect. Interestingly, the combination of both antibodies did not completely reverse CAM-DR. Furthermore, the new small molecule LFA-1 inhibitor LFA703, a statin derivative lacking 3-hydroxy-3-methylglutaryl-coenzyme-A (HMG-CoA) reductase activity,
versus HS-5) of HS-5 stromal cells and PI uptake into CD38 treated for 48 hours with 20

determination may be the cause of CAM-DR inhibition and that targeting these effects. Therefore, we hypothesized that perturbations in signal transduction may be the cause of CAM-DR inhibition and that targeting these effects may reduce the vice versa action for activation. Surprisingly, only GG-PP, not F-PP, was able to prevent simvastatin-induced CAM-DR inhibition. Figure 5A shows

Figure 2. Targeting adhesion molecules may overcome CAM-DR. (A) Blocking monoclonal antibodies against VLA-4 and LFA-1 can overcome CAM-DR. NCI-H929 myeloma cells were treated for 48 hours with 20 μM melphan in the absence or presence (suspension versus HS-5) of HS-5 stromal cells and PI uptake into CD38 cells was determined by flow cytometry. Blocking monoclonal antibodies directed against VLA-4 (4 μg/mL) and LFA-1 (4 μg/mL) were added as indicated in the figure. (B) LFA-1 inhibitor LFA703 can overcome CAM-DR. NCI-H929 myeloma cells were treated for 48 hours with 20 μM melphan in the absence or presence (suspension versus HS-5) of HS-5 stromal cells and PI uptake into CD38 cells was determined by flow cytometry. LFA703 (3 μM) was added as indicated in the figure. (C) CAM-DR reversal is not mediated by de-adhesion. NCI-H929 (NCI) myeloma cells were incubated in the presence (+HS-5) or absence (in suspension) of HS-5 stromal cells and 5 μg/mL anti-VLA4 monoclonal antibody or 3 μM LFA703 were added for 48 hours. The number of viable cells in suspension was determined by the WST-1 viability assay. (D) CAM-DR reversal is not mediated by reduced IL-6 secretion. NCI-H929 and HS-5 were cocultivated as indicated and 5 μg/mL anti-VLA4 monoclonal antibody or 3 μM LFA703 were added. IL-6 concentration in the supernatant was determined by ELISA. Mean values and standard deviations and P values are shown in the figure. n.s. indicates not significant.

HMG-CoA reductase inhibitor simvastatin overcomes CAM-DR

It has been shown that statins modulate integrin function,13 that HMG-CoA reductase inhibitors show antmyeloma activity,14 and that HMG-CoA reductase is differentially up-regulated in adherent, de novo resistant cells.15 In this context we tested the HMG-CoA reductase inhibitor simvastatin in combination with melphan. Figure 3A shows almost complete abrogation of CAM-DR by addition of simvastatin. Thus, simvastatin is much more potent in CAM-DR inhibition than LFA-703. To test whether this is a substance-specific effect and whether other HMG-CoA reductase inhibitors are more potent in CAM-DR inhibition, we evaluated lovastatin in this context. As Figure 3B shows,Lovastatin reduces CAM-DR to levels that are also no longer significantly different from melphan-treated suspension cells. In conclusion, we suggest that HMG-CoA reductase inhibition is sufficient to overcome CAM-DR. Because it was demonstrated previously that statins can down-regulate integrin expression,13 we determined simvastatin-induced de-adhesion but could not detect substantial differences (Figure 3C). Furthermore, IL-6 secretion remains unaltered after simvastatin treatment (Figure 3D). Because the bisphosphonates have been described to have inhibitory effects on the HMG-CoA/cholesterol pathway, zolendronate, pamidronate, and clodronate were tested extensively in this setting. Interestingly, these compounds were not able to restore CAM-DR (data not shown).

The HMG-CoA/GG-PP/Rho/Rho-kinase pathway is crucial for CAM-DR

Because inhibition of HMG-CoA reductase almost completely overcomes CAM-DR in vitro, we addressed the question which downstream molecules may be involved in primary drug resistance. Figure 4 shows a summary of the HMG-CoA pathway. The Ras/Ark/MEK/MAPK pathway is important in multiple myeloma and Ras protein has to be modified posttranslationally by farnesylation for activation. Surprisingly, only GG-PP, not F-PP, was able to prevent simvastatin-induced CAM-DR inhibition. Figure 5A shows
that addition of F-OH, a cell-permeable form of F-PP, did not alter simvastatin-enhanced chemosensitivity, whereas addition of GG-OH, a cell-permeable form of GG-PP, almost completely prevented the simvastatin effect. In concordance, only the geranylgeranyl transferase (GGTase) inhibitor GGTI-298, not the farnesyl transferase (FTase) inhibitor FTI-277, showed statinlike inhibition of CAM-DR (Figure 5B). FTI-277 inhibits Ras processing in whole cells; however, it does not inhibit geranylgeranylation of small G proteins even at high doses. In contrast, GGTI-298 potently inhibits geranylgeranylation of small G proteins by GGTase but has no effect on the processing of H-Ras even at high concentrations. In these very comprehensive experiments melphalan monoculture control is not equal in all motives. This may be due to biologic variations in a setting with several different parameters (confluence of HS-5 monolayer, starvation in wells with low cytotoxicity, cell detritus in wells with high cytotoxicity, etc). We cannot completely exclude substance-specific effects in some motives, but all experiments have been repeated several-fold and we have always indicated that addition of F-OH, a cell-permeable form of F-PP, did not alter simvastatin-enhanced chemosensitivity, whereas addition of GG-OH, a cell-permeable form of GG-PP, almost completely prevented the simvastatin effect. In concordance, only the geranylgeranyl transferase (GGTase) inhibitor GGTI-298, not the farnesyl transferase (FTase) inhibitor FTI-277, showed statinlike inhibition of CAM-DR (Figure 5B). FTI-277 inhibits Ras processing in whole cells; however, it does not inhibit geranylgeranylation of small G proteins even at high doses. In contrast, GGTI-298 potently inhibits geranylgeranylation of small G proteins by GGTase but has no effect on the processing of H-Ras even at high concentrations. In these very comprehensive experiments melphalan monoculture control is not equal in all motives. This may be due to biologic variations in a setting with several different parameters (confluence of HS-5 monolayer, starvation in wells with low cytotoxicity, cell detritus in wells with high cytotoxicity, etc). We cannot completely exclude substance-specific effects in some motives, but all experiments have been repeated several-fold and we have always

![Figure 3. HMG-CoA reductase inhibitor simvastatin overcomes CAM-DR.](image)

Integrin modulator and HMG-CoA reductase inhibitor simvastatin can overcome CAM-DR. NCI-H929 myeloma cells were treated for 48 hours with 20 μM melphalan in the absence or presence (suspension versus HS-5) of HS-5 stromal cells and PI uptake into CD38+ cells was determined by flow cytometry. Simvastatin (10 μM) was added as indicated in the figure. (B) Lovastatin overcame CAM-DR. Experiments were repeated with 1 μM lovastatin as described in panel A. (C) CAM-DR reversal is not mediated by de-adhesion. NCI-H929 (NCI) myeloma cells were incubated in the presence (+HS-5) or absence (in suspension) of HS-5 stromal cells and 10 μM simvastatin was added for 48 hours. The number of viable cells in suspension was determined by the WST-1 viability assay. (D) CAM-DR reversal is not mediated by reduced IL-6 secretion. NCI-H929 and HS-5 were coincubated as indicated and 10 μM simvastatin was added during 48 hours. IL-6 concentration in the supernatant was determined by ELISA. Mean values with standard deviations and P values are shown. n.s. indicates not significant.

![Figure 4. The mammalian mevalonate pathway.](image)

The scheme shows the key signaling molecules and enzymes of the mevalonate pathway. Statins inhibit HMG-CoA reductase, FTI-277 inhibits farnesyl transferase (FTase), GGTI-298 inhibits geranylgeranyl transferase (GGTase). Farnesol (F-OH) is the cell-permeable prodrug of farnesyl pyrophosphate (F-PP) and geranylgeranol (GG-OH) is the cell permeable prodrug of geranylgeranyl pyrophosphate (GG-PP).

![Figure 5. The HMG-CoA/GG-PP/Rho/Rho-kinase pathway is crucial for CAM-DR.](image)

(A) Simvastatin-induced CAM-DR inhibition is mediated via GG-PP. NCI-H929 myeloma cells were grown in the presence (+HS-5) or absence (in suspension) of HS-5 cells. Simvastatin (1 μM) and melphalan (20 μM) were added for 48 hours as indicated. Only GG-OH (10 μM), but F-OH (10 μM), was able to prevent simvastatin-induced CAM-DR inhibition. (B) CAM-DR is mediated via GGTase. Cocultured NCI-H929 (NCI) and HS-5 cells were treated with 30 μM melphalan. GGTI-298 (5 μM), but not FTI-277 (2.5 μM), was able to prevent CAM-DR. (C) CAM-DR is mediated via Rho kinase activation. Cocultured NCI-H929 (NCI) and HS-5 cells were treated with 20 μM melphalan. Inhibition of Rho kinase by 20 μM Y-27632 prevents CAM-DR. (D) Inhibition of the GG-PP/Rho/Rho-kinase pathway does not induce de-adhesion. NCI-H929 (NCI) myeloma cells were grown adherent to HS-5 cells or in suspension as control. Then, 5 μM GGTI-298 or 20 μM Y-27632 was added for 48 hours. The number of viable cells in suspension was determined by the WST-1 viability assay. (E) Inhibition of the HMG-CoA/GG-PP/Rho/Rho-kinase pathway does not induce de-adhesion. NCI-H929 myeloma cells were treated for 48 hours with 1 μM simvastatin, 5 μM GGTI-298, or 20 μM Y-27632. Surface expression levels of the adhesion molecules VLA-4 and LFA-1 were determined by flow cytometry. Mean values with standard deviations and P values are shown. n.s. indicates not significant.
obtained similar results. We can clearly conclude that mainly GG-PP, not F-PP, is involved in CAM-DR signaling. To support this observation we further investigated downstream molecules. Geranylgeranylated Rho protein translocates from cytosol to the membrane and activates Rho kinase. Interruption of this pathway by the Rho kinase inhibitor Y-27632 showed similar inhibition of CAM-DR such as simvastatin (Figure 5C). CAM-DR was completely reversed in comparison to melphalan control in both samples. Because Rho and Rho kinase play a crucial role in cell adhesion and cell migration, the de-adhesion assay was repeated with these 2 compounds. Figure 5D does not demonstrate substantial de-adhesion after GGTI-298 and Y-27632 treatment, again suggesting de-adhesion of myeloma cells not to be the main mechanism of CAM-DR inhibition. Because statins have been shown to down-regulate integrins on lymphocytes, expression of adhesion molecules on myeloma cells on therapy with simvastatin, GGTI-298, and Y-27632 was determined by flow cytometry (Figure 5E). A slight down-regulation of VLA-4 and LFA-1 was seen after simvastatin and GGTI-298, but the alterations did not seem to be strong enough to explain 100% reversal of CAM-DR. Furthermore, after Y-27632 treatment, the expression pattern of adhesion molecules remains unchanged, suggesting the integrin modulatory effect of simvastatin is not mediated by Rho kinase. In conclusion, CAM-DR in multiple myeloma seems to be mediated by activation of the HMG-CoA/GG-PP/Rho-protein/Rho-kinase pathway. Inhibition of this pathway by statins completely overcomes CAM-DR in vitro.

Discussion

Cell adhesion has been elucidated to be the major cause of primary drug resistance in multiple myeloma and many other malignant diseases such as acute and chronic leukemia, lymphoma, lung cancer, and breast cancer. In preliminary experiments we could demonstrate a trend toward reduction of cell death by adhesion of myeloma cells to FN and herewith confirm the results of Damiano and colleagues, but in accordance with the original article there are only slight differences in apoptosis on melphalan treatment between adherent and suspended cells, which do not meet statistical significance in our setting. Little is known about drug resistance induced by adhesion to BMSCs in multiple myeloma. One study shows resistance to mitoxantrone, a compound that is uncommon in the therapy of multiple myeloma. Therefore, we have established an in vitro model for CAM-DR using the human BMSC line HS-5 and different myeloma cells. Strong reduction of chemosensitivity on adherence could be detected for common antitumor agents such as melphalan, treosulfan, doxorubicin, dexamethasone, and bortezomib. Other proteasome inhibitors such as MG-132, which induce apoptosis in multiple myeloma, were similarly much less effective in adherent cells. Although bortezomib has been shown to be a potent drug even in patients resistant to multiple drugs, our observation of reduced efficacy in the coculture system may explain the two thirds of primary nonresponders. In our model all 4 myeloma cell lines showed strong resistance to chemotherapeutic drugs when coincubated with HS-5 BMSCs. Furthermore, we can demonstrate that early apoptosis as well as irreversible cell death is reverted by BMSC contact. As in previous studies, neither transfer of conditioned medium nor sharing the same medium by transwell experiments influenced cell viability, suggesting that direct cell-cell contact is necessary and that soluble factors are not the major mechanism for CAM-DR in multiple myeloma. In this context, it has already been demonstrated that adhesion molecules are up-regulated in drug-resistant cell lines. We expanded on this finding by demonstrating in the current study that stromal cells up-regulate the corresponding adhesion molecules as well, suggesting enhancement of adhesion and herewith enhancement of drug resistance. Interestingly, blocking adhesion by monoclonal antibodies led to a certain but not complete inhibition of CAM-DR in our model. Weitz-Schmidt and coworkers have developed new integrin inhibitors, such as LFA703, that strongly inhibit αβ integrin and herewith the proliferation of T cells. In our experiments LFA703 significantly reduced CAM-DR. Until today this LFA-1 inhibitor has been supposed primarily for the treatment of inflammation by inhibition of leukocytes. Considering the importance of CAM-DR in hematologic and solid neoplasms, our current study suggests for the first time that LFA703 and its derivatives may be promising compounds in cancer therapy. Although LFA703 is a statin derivative, which completely lacks HMG-CoA reductase inhibition activity, and although statins such as lovastatin have been shown to inhibit integrin interaction, reversal of CAM-DR by LFA703 was not complete and the role of HMG-CoA reductase still had to be evaluated.

Statins are widely used in clinical practice because they are effective in the prevention of cardiovascular events. But their ability to reduce atherosclerotic diseases seems to be greater than their ability to lower serum cholesterol by blocking mevalonate synthesis. Studies have shown that statins have direct antioxidant, anti-inflammatory, and antiangiogen properties. Interestingly, simvastatin reduces expression of adhesion molecules in circulating monocytes from hypercholesterolic patients. Similarly, in our experiments simvastatin reduced the level of LFA-1 and VLA-4 expression on multiple myeloma cells. Statins also inhibit HMG-CoA reductase, which is the rate-limiting enzyme of the cholesterol pathway. Activation of this pathway leads to the production of intermediates such as GG-PP. GG-PP activates Rho by posttranslational modification, a process that is inhibited by statins. Mevastatin, for example, inhibits bone resorption and induces osteoclast apoptosis in vitro by inhibition of protein prenylation. Furthermore, statins have been shown to induce apoptosis in melanoma, thyroid cancer, colon carcinoma, squamous cell carcinoma, breast cancer, acute myeloid leukemia, malignant lymphoma, and multiple myeloma cells in vitro. In our experiments, statins have been used in much lower concentrations than in most in vitro studies published with complete reversal of CAM-DR, suggesting realistically achievable doses in humans. Initially, we tested a broad variety of pathway modulators, which are critically involved in multiple myeloma, but did not observe any substantial effect. In the light of this large series of experiments the efficacy of statins in CAM-DR reversal seems exceptional. With exception of the toxicity data, not much is known about the role of the mevalonate pathway in multiple myeloma. In support of our hypothesis about the involvement of the mevalonate pathway in CAM-DR, very recently oligonucleotide microarray analyses demonstrated that de novo and acquired drug resistance are associated with an increase of HMG-CoA reductase gene expression.

Bisphosphonates interrupt the mevalonate signaling pathway and induce apoptosis in vitro and in vivo. Inosoprenylation of Rap1A protein has been shown to be reduced in myeloma cells...
after bisphosphonate therapy. Premylation changes of Rho protein have not been described. We have extensively tested several bisphosphonates using many different incubation periods and concentrations, but did not observe inhibition of CAM-DR. We conclude that mevalonate subpathways other than GG-PP/Rho/Rho kinase are involved in bisphosphonate-induced apoptosis and that inhibition of these pathways does not contribute to CAM-DR inhibition.

Small G proteins (also known as small GTPases, small GTP-binding proteins, and Ras protein superfamily) are monomeric molecules with molecular weight of 20 to 40 kDa that bind and hydrolyze guanine nucleotides. Because their GTPase activity mediates the termination of their function, but actually not their function, the term small G protein is used here. There are about 150 known small G proteins, which have been divided into 5 families (Ras, Rho, Rab, Sar1/Arf, and Ran). They regulate a wide variety of cell functions as well as the regulation of protein translocation from the cytosol to the membrane. In contrast to this, Ras and activation of geranylgeranylated proteins have not been investigated. Therefore, the functional relevance of these data remains to be determined and geranylgeranylation could be a further important mechanism in this model.

Germanskylation of Rho protein leads to activation of this small G protein. Rho interacts with several effectors such as Rho kinase, protein kinase N, rhokinetic, citron, p140mDia, and phospholipase D. Rho kinase is one of the best-characterized Rho effectors. Activation of RhoA or Rho kinase has been shown to increase invasiveness of rat hepatoma cells, whereas inhibition of Rho kinase can reduce tumor growth and metastasis in different models. Our efforts to overcome CAM-DR revealed high efficacy of the Rho kinase inhibitor Y-27632. This compound almost completely restored chemosensitivity. Therefore, we conclude that germanskylation of Rho protein with consequent activation of Rho kinase mediates CAM-DR and interruption of this pathway provides new molecular targets for the therapy of multiple myeloma. This is in concordance with the observation in thyroid cancer that Rho protein is the pivotal element for cellular survival, whereas farnesylation of Ras proteins was not involved. Interestingly, the isoprenylated proteins did not influence the cytostarvation of the cells, because RhoA has been implicated in changes of cell morphology, formation of stress fibers, and focal adhesion. The roles of Rho protein and Rho kinase in multiple myeloma are poorly understood. Recently, it has been shown that the Rho kinase inhibitor responsible for Wnt-induced morphologic changes in myeloma cells and that the Rho kinase inhibitor Y-27632 strongly reduces IGF-1–induced migration of myeloma cells.

In conclusion, our work has elucidated that the HMG-CoA/PP/Rho/Rho kinase pathway mediates CAM-DR in multiple myeloma cells. Inhibition of this pathway by statins, which are widely used in patients, can completely overcome drug resistance. Because this effect can be observed at very low, nontoxic concentrations of statins, the combination of HMG-CoA reductase inhibitors with conventional chemotherapeutic agents needs to be evaluated in a clinical setting. Furthermore, in this study we show for the first time that the in vitro efficacy of selective integrin inhibitors such as LFA703 in cancer therapy. Because CAM-DR can be observed in many hematologic and solid malignancies and because statins have been shown to influence cell survival in a broad variety of neoplasms in vitro, the concomitant targeting of CAM-DR by statins during chemotherapy may become an important strategy in many malignant diseases, not only multiple myeloma.

References


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