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Inhibition of the mevalonate pathway affects epigenetic regulation in cancer cells

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The mevalonate pathway provides metabolites for post-translational modifications such as farnesylation, which are critical for the activity of RAS downstream signaling. Subsequently occurring regulatory processes can induce an aberrant stimulation of DNA methyltransferase (*DNMT1*) as well as changes in histone deacetylases (HDACs) and microRNAs in many cancer cell lines. Inhibitors of the mevalonate pathway are increasingly recognized as anticancer drugs. Extensive evidence indicates an intense cross-talk between signaling pathways, which affect growth, differentiation, and apoptosis either directly or indirectly via epigenetic mechanisms. Herein, we show data obtained by novel transcriptomic and corresponding methylomic or proteomic analyses from cell lines treated with pharmacologic doses of respective inhibitors (i.e., simvastatin, ibandronate). Metabolic pathways and their epigenetic consequences appear to be affected by a changed concentration of NADPH. Moreover, since the mevalonate metabolism is part of a signaling network, including vitamin D metabolism or fatty acid synthesis, the epigenetic activity of associated pathways is also presented. This emphasizes the far-reaching epigenetic impact of metabolic therapies on cancer cells and provides some explanation for clinical observations, which indicate the anticancer activity of statins and bisphosphonates.

Keywords Mevalonate pathway, statins, bisphosphonates, epigenetics, cancer metabolism

For more than 100 years, it has been known that cholesterol may accumulate in cancerous tissues (1) and plays a critical role in cancer progression, thus emphasizing the therapeutic potential of lowering cholesterol and downregulating the mevalonate pathway in cancer prevention and treatment (2). The mevalonate pathway converts acetyl-coenzyme A (acetyl-CoA) to isoprenoids, thus supplying key metabolites for cholesterol and steroid synthesis. It comprises a series of enzymatic reactions that occur in the endoplasmic reticulum. The rate-limiting step is catalyzed by 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, which converts HMG-CoA to mevalonate. This reaction is inhibited by statins, whereas bisphosphonates target more downstream reactions in this pathway, such as farnesylation and geranylgeranylation.

Meanwhile, there exists an increasing amount of data, which indicate that statins, as well as bisphosphonates, target the three most important epigenetic levels: DNA methylation, histone deacetylation, and microRNAs (Figure 1).

The best-described epigenetic roles of statins and bisphosphonates result from a reduction of the membrane anchoring from RAS and associated signaling toward DNA

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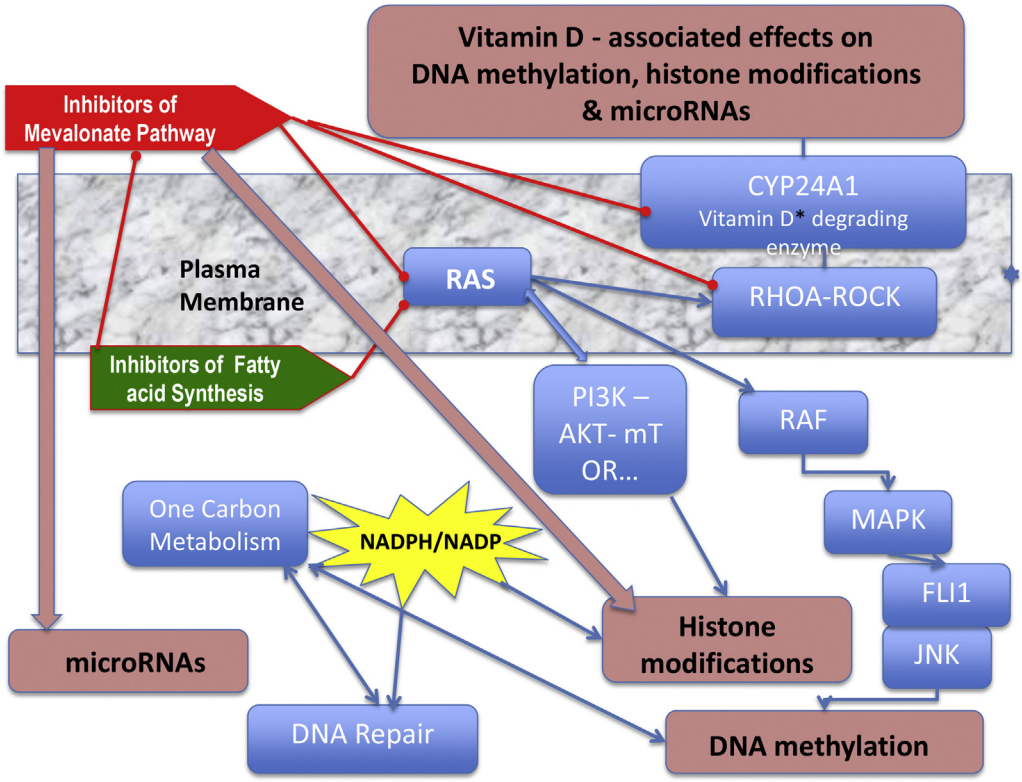

Figure 1 Inhibition of the mevalonate pathway influences the stability of the plasma membrane. It inhibits isoprenylation of the small GTP-binding proteins and, therefore, the activity of RAS signaling. As a consequence, RAS signals via RAF into the MAPK pathway, an inhibited signaling via FLI1 and JNK (c-JUN N-terminal kinase), leads to a downregulation of DNMT1. The cross-talk of RAS with PI3K-AKT-mTOR signaling influences the expression of HDACs. Additional metabolic pathways influenced by RAS signaling are glucose uptake and the OCM, which may both be fueled by activating mutations of the P53 gene (*TP53*) and play essential roles in DNA repair and inflammation. Similar to the inhibition of HMG-Co-A reductase, a downregulation of these pathways changes the concentration of NADPH. In addition, there is also a downregulation of the RHOA-ROCK signaling and the associated vitamin D degrading enzyme CYP24A1 (18). This could induce a series of vitamin D–associated effects on fatty acid metabolism and epigenetics, for example (13).

demethylation (3,4), or downregulation of the histone deacetylase HDAC2 via the RAS/PI3K/mTOR pathway (5) in addition to a direct competitive inhibition of HDAC2 by statins (6). Reduction of homocysteine, which is produced in the one carbon metabolism (OCM), also leads to a downregulation of the DNA methyltransferase DNMT1 (7) and a shift in the NAD(P)$^+$/NAD(P)H-ratio toward NADP, with apparent consequences for histone modifications (8–10) and DNA repair through breakdown of poly-ADP-ribose (9). The downregulation of geranylgeranylation of another small GTPase, RHOA, and associated signaling (11) downregulates HDAC1 (12) and promotes vitamin D–associated epigenetic effects (13–15) by preventing CYP24A1-induced degradation of vitamin D3 (16–18).

In this study, simvastatin was chosen as a representative statin for transcriptomic studies, because a large-scale investigation was already performed with this drug and it was the first statin drug used extensively in clinical practice for control of elevated cholesterol. Epigenetic studies with simvastatin emphasize its role as a direct inhibitor of HDAC1 and HDAC2 (6) or as an inducer of respective microRNAs (19–21). Ibandronate was selected as a representative bisphosphonate, because it is already known for its epigenetic impact (3).

Materials and methods

Cell cultivation treatment and NADP$^+$/NADPH analyses

Cells were cultivated in cell culture flasks at 37°C and 5% CO_2. The culture media were as recommended by the American Type Culture Collection (ATCC) for MDA-MB-231 breast cancer DMEM (Sigma-Aldrich, St. Louis, MO, USA), which contained 10% fetal calf serum (FCS); PC-3 prostate carcinoma DMEM-F12 (Sigma-Aldrich) with 10% FCS. MG-63 and U2-OS osteosarcoma were cultured in AlphaMEM (Biochrom, Berlin, Germany) medium containing 10% FBS. For the HMC1.1 cell line, we used Iscove's Modified Dulbecco's Medium (IMDM; Thermo Fisher Scientific, Waltham, MA) supplemented with 260 nM thioglycerol (Sigma-Aldrich) and 20% fetal bovine serum (FBS). All culture media contained 10 μg/mL gentamycin (Sigma-Aldrich). To guarantee optimal growth, cells were split two times a week and reseeded at a density of $2–5 \times 10^5$ cells/mL.

One day after splitting, 32 μM simvastatin (Sigma-Aldrich) or 150 μM ibandronate (Sigma-Aldrich) were added to the culture