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Expression of Prelamin A Confers Sensitivity of DNA Biosynthesis to Lovastatin on F9 Teratocarcinoma Cells

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Expression of prelamin A but not mature lamin A confers sensitivity of DNA biosynthesis to lovastatin on F9 teratocarcinoma cells

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SUMMARY

The role of inhibition of prelamin A processing in the inhibition of DNA synthesis by lovastatin was examined by expressing prelamin A in F9 teratocarcinoma cells. These cells, normally lacking expression of the A/C lamins, were transfected with constructs expressing either prelamin A or mature lamin A and the effect of lovastatin on DNA biosynthesis was assessed. It was found that expression of prelamin A specifically conferred sensitivity to inhibition of DNA biosynthesis by lovastatin on F9 cells.

Key words: protein prenylation, lovastatin, lamin A, DNA synthesis, prelamin A

INTRODUCTION

It has been known for some time that starvation of mammalian cells for mevalonate produces an inhibition of DNA biosynthesis and an eventual cell cycle arrest (Quesney-Huneeus et al., 1979). Initial studies on a few cell lines showed that inhibition of mevalonate biosynthesis by means of the 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (Habernicht et al., 1980; Fairbanks et al., 1984), compactin or lovastatin, or by mutation of HMG-CoA synthase (Sinensky and Logel, 1985) resulted in blocks early in G1, and later studies extended this observation to a large number of permanent cell lines (Keyomarski et al., 1991). The discovery of proteins post-translationally modified with the isoprenoids farnesyl and geranylgeranyl (Schmidt et al., 1984; Rilling et al., 1990) led to the suggestion (Sinensky et al., 1985) that a decline in level of the prenylated form of one of these proteins during mevalonate starvation might be responsible for the cell cycle arrest.

Intriguing candidates for this role are the farnesylated, p21ras proteins. However, studies with cells expressing a mutant p21ras, whose biological activity is farnesylation-independent, showed that these cells still exhibited sensitivity of DNA synthesis to mevalonate starvation (DeClue et al., 1991).

Another possible candidate protein, which could be responsible for the inhibition of DNA biosynthesis that follows mevalonate starvation, is prelamin A. We have previously shown that prelamin A undergoes a farnesylation-dependent removal of a carboxyl-terminal peptide (Beck et al., 1990) that blocks assembly of lamin A into the nuclear lamina (Lutz et al., 1992). Remarkably, lamin A molecules lacking this carboxyl-terminal domain are competent for assembly into the lamina. The lack of a functional requirement for farnesylation in assembly, other than to remove a domain inhibitory for assembly, suggests that this processing event may be of regulatory significance.

Cells treated with lovastatin accumulate prelamin A in the nucleoplasm (Lutz et al., 1992), an observation that was previously seen with the mutant prelamin A that is incompetent for farnesylation (Holtz et al., 1989). Considering the proposed interaction between the nuclear lamina and chromatin in the interphase nucleus (Burke, 1990; Glass and Gerace, 1990; Paddy et al., 1990; Yuan et al., 1991), it is a plausible hypothesis that this mislocalized prelamin A could be responsible for some of the effects of mevalonate starvation on DNA synthesis.

To examine the role of prelamin A in the loss of DNA synthesis in response to mevalonate starvation it would be useful to compare isogenic cell lines that express or do not express prelamin A. A model for this situation has been described that takes advantage of the lack of expression of the A/C lamin proteins in stem cell tumor lines such as the mouse F9 teratocarcinoma (Lebel et al., 1987). The F9 cell has been transiently transfected with the human prelamin A cDNA and demonstrated to assemble this heterologous lamin A into the nuclear lamina (Collard and Raymond, 1990). In this report we describe the isolation of F9 cell lines that possess dexamethasone-inducible prelamin A or mature lamin A. The effects of prelamin A accumulation in cells starved for mevalonate by lovastatin treatment can now be determined relative to cells expressing only mature lamin A. The results are consistent with a substantial increase in sensitivity of cellular DNA synthesis to mevalonate starvation when prelamin A accumulation can occur in response to lovastatin treatment.

MATERIALS AND METHODS

Cells, transfections and media
The cells used in these studies were F9 mouse teratocarcinoma cells (ATCC CRL 1720). Cells are grown in DMEM supplemented with 10% fetal calf serum or charcoal-stripped fetal calf serum. These cells have been reported previously to lack detectable expression of the
Effect of lovastatin on cholesterol biosynthesis

F9 cells were inoculated at a density of 1×10⁶ cells/60 mm culture dish. Cells were incubated in various concentrations of lovastatin for 1 hour and then 20 µCi of [³H]acetate (sodium salt) (DuPont-New England Nuclear, Waltham, MA; specific activity = 22 Ci/mmol) was added per plate. The cells were incubated for 17 hours and the lipids extracted with hexane/isopropyl alcohol (3:2, v/v), saponified and the non-saponifiable material was isolated as described previously (Panini et al., 1989). Radioactive cholesterol was isolated by thin-layer chromatography on silicic acid-coated plates (LK5D, Whatman, Hillsboro OR), developed with petroleum ether:acetone (90:10, v/v) and visualized by fluorography after spraying with ENHANCE (New England Nuclear, Waltham, MA). The radioactive cholesterol bands were scraped from the plates into scintillation vials and quantified by liquid scintillation counting.

Immunoprecipitation of p21ras

Cell labeling with ³⁵S (Tran³⁵S-label, specific activity ≥1000 Ci/µmol (ICN, Costa Mesa, CA); harvesting and lysis were essentially as described previously (Leonard et al., 1990) except for the culture medium being DMEM. Ras protein was immunoprecipitated as described previously (Leonard et al., 1990) except that a different pan Ras mouse monoclonal antibody (ab3, Oncogene Sciences, Uniondale NY) was used.

Rate of DNA biosynthesis

Cells (2.5×10⁵) were incubated for 1 hour with 1 µCi/ml of [methyl-³H]thymidine (ICN, Irvine CA; specific activity = 20 Ci/µmol). The label incorporated into DNA was determined by precipitation with ice-cold trichloroacetic acid, collection of the precipitate on GFA, glass fiber filters (Whatman, Hillsboro OR) and quantification by liquid scintillation counting. Protein was determined by the method of Schacterle and Pollack (1973).

RESULTS

The sensitivity of mevalonate biosynthesis to lovastatin in F9 cells is comparable to that in other mammalian cell lines but that of DNA biosynthesis is not

Lovastatin is a potent inhibitor of mevalonate biosynthesis in most cultured mammalian cells with an IC₅₀ of less than 10⁻⁸ M (~4 ng/ml), based on the incorporation of labeled acetate into cholesterol. We examined the effect of lovastatin on the incorporation of acetate into cholesterol in F9 cells. We observed that neither the uptake of lovastatin nor its inhibition of HMG-CoA reductase is less efficient than that observed for other mammalian cells.

We also determined the activity of lovastatin as an inhibitor of protein prenylation by examining its effects on the processing of p21ras. The results (Fig. 2) indicate that a concentration of 1 µg/ml lovastatin produces a substantial inhibition of p21ras processing in F9 cells, which is comparable to its effects on the processing of p21ras in other cell lines we have examined (Sinensky et al., 1990).

Most mammalian cells studied thus far also exhibit an inhibition of DNA biosynthesis in response to treatment with lovastatin (Keyomarski et al., 1991). However, despite the sensitivity of mevalonate biosynthesis in F9 cells to lovastatin comparable to that seen with other cell lines (the CHO-K1 cell line, for example), we find that F9 cells are remarkably resistant to inhibition of DNA biosynthesis by lovastatin (Fig. 3). CHO-K1 cells, like F9 cells, have an IC₅₀ for inhibition of acetate incorporation into cholesterol of ~5 ng/ml and show marked inhibition of labeled methionine incorporation into mature p21ras when treated with 1 µg/ml lovastatin (Sinensky et al., 1990).

Expression of prelamin A but not mature lamin A renders F9 cellular DNA synthesis sensitive to lovastatin

We wished to test the hypothesis proposing that the lack of sensitivity of F9 cells to inhibition of DNA biosynthesis by lovastatin might be due to the absence of accumulation of prelamin A in these cells during the treatment with lovastatin.
Therefore, we transfected F9 cells with a prelamin A construct that we have described previously (Sinensky et al., 1994), which permits the dexamethasone induction of prelamin A expression in transfected cells. Since the nucleoplasmic localization of prelamin A is farnesylation-dependent (Lutz et al., 1992), we also wished to determine whether there was a difference in response to lovastatin by cells expressing only mature lamin A as opposed to those expressing only the prelamin A precursor. Thus, we also prepared transfectants that express a dexamethasone-inducible mature lamin A using another previously described construct, pMMLAΔ21 (Lutz et al., 1992). Cells were transfected with the pMMLA and pMMLA Δ21 constructs, co-transfected with the selectable neoR gene and the resultant G418-resistant clones were screened by indirect immunofluorescence with an anti-A/C lamin antiserum. Immunofluorescence detection of transfectants is demonstrated in Fig. 4. As we have previously reported for both pMMLA and pMMLAΔ21 (Sinensky et al., 1994; Lutz et al., 1992), no expression was detected by indirect immunofluorescence in cells transfected with these constructs in the absence of dexamethasone treatment (data not shown).

We now examined these transfectants that express prelamin A or mature lamin A for sensitivity of DNA synthesis to lovastatin. The results (Fig. 5) are consistent with an increase in the sensitivity of DNA synthesis to lovastatin relative to the parental F9 cells in cells that express prelamin A (F9LA) but not in those that express mature lamin A (F9LAΔ21). Control experiments demonstrated that dexamethasone treatment had no effect on DNA synthesis in F9 cells or on DNA synthesis in F9LA cells in the absence of lovastatin (data not shown).

**DISCUSSION**

It has been observed for many cell types that lovastatin treatment produces an inhibition of cell growth. For cells growing in the presence of serum lipoproteins, this phenomenon has been ascribed to a deficit in protein prenylation (Sinensky and Logel, 1985). The data reported here are consistent with the hypothesis that, at least in part, the inhibition of DNA biosynthesis observed in lovastatin-treated cells is due to the accumulation of unprocessed prelamin A.

Fig. 3. Effect of lovastatin (1 µg/ml) on the rate of DNA synthesis in F9 cells. Cells are incubated with [3H]thymidine for 1 hour after incubation for 28 hours in the presence (+) or absence of lovastatin (−). Incorporation of [3H]thymidine into DNA is measured as trichloroacetic acid-insoluble material. A lovastatin responsive cell-line (CHO-K1) is shown for comparison.

Fig. 4. Expression of human lamin A and prelamin A in F9 cells. Clones expressing a construct encoding mature lamin A (B) and prelamin A (C) under control of a dexamethasone-inducible promotor (MMTV) are visualized by indirect immunofluorescence with an anti-lamin A/C antibody as described in Materials and Methods. Non-transfected F9 cells are shown for comparison (A). Cells in all three experiments were treated with 10−7 M dexamethasone, which induces the lamin protein in the transfectants.
assembled into the lamina but rather is localized to the nucleoplasm. It is intriguing to speculate that this mislocalization of prelamin A may be responsible for its effects on DNA biosynthesis. If prelamin A accumulation blocks cells in G1, it might do so by inhibiting the activity of protein factors, such as cyclins, that regulate G1 traversal (Sherr, 1993). On the other hand, prelamin A accumulation might directly inhibit DNA biosynthesis through effects on DNA replication factors and, hence, block cells in S-phase. In either case, it is possible that the inhibition of DNA synthesis produced by prelamin A accumulation may be of regulatory significance and help to explain the puzzling functional role of farnesylation in prelamin A processing.

These observations regarding the effects of prelamin A accumulation on DNA biosynthesis should also be considered in another context. It has recently been suggested that farnesyl protein transferase inhibitors might be useful as anti-malignancy compounds through their activity in blocking the processing of oncogenic pro-p21ras (Kohl et al., 1993; James et al., 1993). In light of the studies reported herein, it would be of interest to examine the effects of these compounds on prelamin A processing, since the inhibition of DNA biosynthesis, produced by the accumulation of prelamin A, might contribute to the efficacy of such compounds in blocking cellular proliferation.

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REFERENCES


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