Functional Interactions between Bile Acids, All-Trans Retinoic Acid, and 1,25-Dihydroxy-Vitamin D₃ on Monocytic Differentiation and Myeloblastin Gene Down-Regulation in HL60 and THP-1 Human Leukemia Cells

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ABSTRACT

Bile acids were shown previously to inhibit proliferation and to induce monocytic differentiation in HL60 human acute promyelocytic leukemia cells (A. Zimber et al., Int. J. Cancer, 59: 71–77, 1994). In this report, we hypothesized that bile acids may exert a positive cooperativity with two known inducers of leukemia cell differentiation, all-trans retinoic acid and 1,25(OH)₂ vitamin D₃. Our results provide evidence that bile acids induced the monocytic differentiation of HL60 and THP-1 human leukemia cells exposed to ineffective concentrations of these inducers. The protein kinase C (PKC) inhibitors H-7 (10 and 20 μM) and staurosporine (5 and 20 nM) modulated the effects of bile acids on HL60 cell differentiation. Most interestingly, bile acids are shown herein to down-regulate the expression of the serine protease myeloblastin gene involved in the differentiation of myeloid hematopoietic cells.

In agreement with the recent identification of nuclear receptors for bile acids, our data suggest that functional interactions between nuclear bile acid signaling pathways, PKC, and nuclear receptors for retinoic acid and vitamin D₃ are involved in the down-regulation of the myeloblastin gene and the induction of cell differentiation in human leukemic cells.

INTRODUCTION

Differentiation-inducing drugs are often used in combination with other compounds that either induce cellular differentiation or exert cytostatic or cytotoxic effects (1, 2). They may then act in an additive or even synergistic manner (3). In such combination therapy, drugs are used simultaneously or sequentially, even at lower concentrations that are not effective or only slightly effective when administered alone, to obtain a significant therapeutic gain, e.g., inhibition of tumor cell proliferation and induction of differentiation. Practically, this means that side effects may be reduced considerably without losing the desired maximal therapeutic effect of the drugs (4).

Previously, we have shown that several primary and secondary bile acids can inhibit the proliferation and induce maturation-differentiation in HL60 human acute promyelocytic leukemia cells in vitro (5). Thus, after 3–5 days of treatment, these cells were engaged in the monocytic pathway of maturation and differentiation, as judged by morphological examination, NBT3 test, and binding of monoclonal antibodies specific for cell surface differentiation antigens. At the same time, cell cycle analysis showed a very significant accumulation of HL60 cells at the G0–G1 boundary, with a concomitant decrease in the percentage of cells at the S phase (5).

Because the concentrations of bile acids we used to induce the differentiation of HL60 cells (50–100 μM) were similar to serum levels in patients with cholestasis of pregnancy or bile acid concentration in portal blood (6, 7), we decided to extend this study by testing the hypothesis that bile acids may cooperate with well-known natural inducers of differentiation in HL60 and THP-1 cells, along the monocytic or granulocytic pathways. For this purpose, we used ATRA and Vit D₃ at different concentrations, including noneffective ones. Both inducers are physiological and therapeutically active compounds, already used successfully in the treatment of acute promyelocytic leukemia and other diseases (1, 8–10).

We observed that bile acids cooperated with low and physiological concentrations of ATRA and Vit D₃ that are not effective on HL60 cell proliferation and differentiation. We also show that bile acids, as well as ATRA and Vit D₃, caused a down-regulation of the Mbn gene encoding a serine protease directly involved in normal myelopoiesis (11). Because bile acids such as chenodeoxycholic and ursodeoxycholic acid are already used successfully in the treatment of gallstones and cholestatic liver diseases with minor side effects, the results reported herein may have important clinical applications. Also, derivatives of bile acids might be designed with higher efficiency and lower cytotoxicity.

MATERIALS AND METHODS

Cell Culture. HL60 human acute promyelocytic leukemia cells and THP-1 human monocytic leukemia cells were obtained originally from Dr. T. Breitman (National Cancer Institute, Bethesda, MD). HL60 and THP-1 cells were routinely passaged every 3 or 4 days in RPMI 1640 (Life Technologies, Inc., Paisley, United Kingdom) supplemented, respectively, with 10 and 5% FCS (Boehringer, Mannheim, Germany), 2 mM glutamine, penicillin (100 IU/ml), and streptomycin (100 IU/ml), all from Flow (Irvine, United Kingdom). Cells between passages 35 and 60 were used for all experiments described herein.

For testing, the effects of bile acids or other drugs, aliquots of 2–3 × 10⁵ cells taken at days 3 or 4 after passage, were suspended in the standard culture medium. Incubation was performed at 37°C in a humidified atmosphere, with 5% CO₂ in air.

Drugs and Treatments. PMA and the bile acids DCA, CDCA, and LCA were from Sigma Chemical Co. (La Verpillière, France). ATRA and Vit D₃ were kind gifts from Hoffmann-LaRoche (Basel, Switzerland). The PKC inhibitors H-7 and staurosporine were from Calbiochem (La Jolla, CA).

LCA, PMA, and ATRA were first dissolved in DMSO; Vit D₃ stock solution was in absolute ethanol, and all other drugs were dissolved in sterile 0.9% saline solution. All stock solutions were kept at −70°C, further diluted in RPMI just before use, and routinely added to cell suspensions under subdued light. Final concentrations of DMSO and ethanol did not exceed 0.1 or 0.01%, respectively, and these vehicle solvents had no effect on any of the parameters studied. Thus, one pooled control value is given in all tables and figures.

Cell Proliferation and Differentiation. Drugs were added to 25-cm² Falcon flasks (Oxand, CA) containing 10 ml of HL60 or THP-1 cell suspensions. When tested in combination, drugs were added simultaneously, whereas bile acids were always added 15–30 min before retinoic acid or Vit D₃. PKC inhibitors were added one (day 0) or repeatedly, as indicated. Leukemic cells were incubated for various periods of time, usually for 3 or 5 days. At the end of incubation, cell viability was determined by the trypan blue dye exclusion method. Aliquots containing about...
10^6 HL60 cells were then taken for morphological examination, and their distribution in different stages of maturation and differentiation, and for the NBT and NSE tests for cell differentiation, as described previously (5).

Cell Cycle Cytometry. The distribution of HL60 cells among the G0-G1, S, and G2 + M phases of the cell cycle was estimated by fluorescence activated cell sorter, following DNA binding with propidium iodide, as described previously (5).

RNA Isolation and Northern Blot Analysis of Mbn Gene Expression. Total RNA was isolated according to the procedure of Chirgwin et al. (12). RNA blots were obtained after electrophoretic separation in denaturing formamide/agarose gels and transfer onto nylon membranes. RNA bands were visualized by autoradiography, following hybridization with 32P-labeled probes. A random priming Mbn cDNA probe corresponding to positions 260–630 of the Mbn sequence was described previously (11). An even loading of cellular RNA on the gels was ascertained by 28S RNA controls, with ethidium bromide staining.

Statistical Analysis. Student’s t test, ANOVA, and the Mann-Whitney nonparametric ranking test (one-tailed) and linear regression were performed where appropriate (13).

RESULTS

Effect of Combination between DCA and ATRA or Vit D₃ on HL60 Cell Proliferation and Differentiation. We studied the effect of DCA, ATRA, and Vit D₃, each drug alone and in combination. We used DCA at four concentrations: 50 μM, which alone did not affect significantly cell proliferation and differentiation; 75 μM, which was only slightly effective; 100 μM, an effective and subtoxic concentration; and 150 μM, an effective but toxic one. ATRA and Vit D₃ were used in a low concentration of 1 nM, which alone had no effect, and a high concentration of 10 nM, which when administered alone caused significant inhibition of cell proliferation and induced differentiation.

Results presented in Table 1 show that all combination treatments with 100 μM DCA plus ATRA or Vit D₃ were by far superior to the single treatments in the induction of HL60 cell differentiation. In general, the inhibition of cell proliferation paralleled this effect. The combination of 50 μM DCA, alone having no effect on cellular differentiation, showing only 5% NBT-positive cells, with ATRA at 1 and 10 nM, resulted in 22 and 80% NBT-positive cells, respectively. This low concentration of DCA, when used in combination with 10 nM Vit D₃, gave 91% NBT-positive and 59% NSE-positive cells, whereas Vit D₃ alone showed only 50 and 13% positive cells, respectively (data not shown).

The combination of 75 μM DCA (which alone had no effect, showing 6% NBT-positive cells), together with the non-effective concentration of 1 nM ATRA, or 1 nM Vit D₃, resulted in 90 and 70% NBT-positive cells, respectively, reflecting a high degree of cell maturation and differentiation (data not shown).

Table 1 Effect of DCA, ATRA, and Vit D₃ and their combinations on the proliferation and differentiation of HL60 cells.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cell count × 10^6/ml</th>
<th>Cell viability, %</th>
<th>NBT-positive cells, %</th>
<th>NSE-positive cells, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.91</td>
<td>97</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>DCA, 100 μM</td>
<td>1.23</td>
<td>97</td>
<td>52</td>
<td>5</td>
</tr>
<tr>
<td>ATRA, 1 nM</td>
<td>3.18</td>
<td>98</td>
<td>5</td>
<td>ND</td>
</tr>
<tr>
<td>ATRA, 10 nM</td>
<td>1.21</td>
<td>80</td>
<td>70</td>
<td>100</td>
</tr>
<tr>
<td>Vit D₃, 1 nM</td>
<td>2.20</td>
<td>97</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Vit D₃, 10 nM</td>
<td>2.04</td>
<td>92</td>
<td>50</td>
<td>13</td>
</tr>
<tr>
<td>Combinations: DCA, 100 μM plus ATRA, 1 nM</td>
<td>1.53</td>
<td>96</td>
<td>91</td>
<td>ND</td>
</tr>
<tr>
<td>ATRA, 10 nM</td>
<td>0.98</td>
<td>77</td>
<td>98</td>
<td>ND</td>
</tr>
<tr>
<td>Vit D₃, 1 nM</td>
<td>0.84</td>
<td>90</td>
<td>97</td>
<td>52</td>
</tr>
<tr>
<td>Vit D₃, 10 nM</td>
<td>0.73</td>
<td>87</td>
<td>100</td>
<td>73</td>
</tr>
</tbody>
</table>

# # Significantly different from: * nontreated controls (P ≤ 0.05, Mann-Whitney non-parametric test, one-tailed); # # relevant single treatment (P ≤ 0.05). ND, not done.

Treatment with DCA at 150 μM (alone giving 63% NBT-positive cells), in combination with 1 nM ATRA, increased the number of NBT-positive cells to 91%. Because this concentration of DCA was toxic, it was not used further. In contrast, 75 μM DCA was never toxic when used as a single drug or in any combination treatment. This concentration was therefore chosen for additional experiments on cell morphology and cell cycle.

Combination of CDCA with ATRA or Vit D₃. We showed that CDCA inhibited HL60 cell proliferation and induced differentiation (5), and because it is used clinically in the treatment of gallstones and in cholestatic liver diseases with only minor side effects, we tested its effect in combination with either ATRA or Vit D₃.

Results presented in Table 2 show that the combination of 60 μM CDCA (a treatment that alone had no effect), together with either ATRA or Vit D₃, significantly enhanced cellular differentiation (NBT test), and that low concentrations of ATRA and Vit D₃ could be used to obtain such results. In general, the inhibition of cell proliferation correlated well with the differentiation-inducing effect of the drug combinations used. Cell viability was >95% in all of these treatments, showing that none of them was cytotoxic. An isobologram presentation (4) of data, shown here together with our unpublished results on the induction of HL60 cell differentiation (NBT test) by bile acids, alone and in combination with Vit D₃, showed that these interactions were additive (Fig. 1).

Combination of DCA with PMA. PMA was shown previously to induce the differentiation of HL60 cells toward macrophages (14). Both PMA and bile acids were shown to affect PKC activity (15–17) and to act as tumor promoters (18, 19). We have, therefore, tested the effect of their combinations in HL60 cells.

Treatment of HL60 cells with 0.01 or 0.1 nM PMA had no significant effect on the number of NBT-positive cells (0–1%), whereas DCA (50 μM) alone induced only 20% NBT-positive cells. In contrast, the combinations between DCA and these two PMA concentrations increased the number of NBT-positive cells to 35 and 75%, respectively, and enhanced the antiproliferative effect. Thus, results obtained for 0.1 nM PMA plus DCA were almost as pronounced as those obtained by 1 nM PMA alone, corresponding to 84% NBT-positive cells. On the basis of these results and further combinations studied, we concluded that PMA and DCA acted at best in an additive manner, perhaps because both drugs act on PKC or another common target.

Effect of Combination of Bile Acids DCA and CDCA with ATRA or Vit D₃ on the Morphological Maturation and Differentiation of HL60 Cells. To substantiate the beneficial effect of the combinations of bile acids with ATRA and Vit D₃ on HL60 cellular differentiation that was apparent in the NBT test (Tables 1 and 2) or NSE test (Table 1), we have examined the Wright-Giemsa-stained
Bile Acids, ATRA, Vit D₃, and Differentiation in HL60

Effect of Combinations between DCA, ATRA, and Vit D₃ on the Cell Cycle. When bile acids were administered in effective concentrations causing differentiation in HL60 cells, a significant G₀-G₁ cell cycle arrest occurred (5). Here, we tested the effect of combinations of DCA and ATRA or Vit D₃ at concentrations that alone caused only slight effect on cellular differentiation and the cell cycle. This bile acid was used in a concentration of 75 μM, which had no effect by itself, and in an effective concentration of 100 μM. After 5 days of treatment with 75 μM DCA combined with 2 nM of ATRA, there was a significant accumulation of cells at G₀-G₁ phase, from 50 to 76% (Fig. 3). When DCA was added in a dose that alone resulted in the accumulation of 76% of cells at G₀-G₁, its effect was enhanced by the combination with 2 nM Vit D₃, reaching a value of 85% of cells in G₀-G₁ (data not shown). Also, the combination between 100 μM DCA with either ATRA or Vit D₃ accelerated the accumulation of cells at G₀-G₁, which was already 73 and 76% at 3 days, as compared with 62% obtained with DCA alone (data not shown).

Effect of Combinations between Bile Acids and ATRA on the Differentiation of THP-1 Human Leukemia Cells. As shown in Fig. 4, treatment of THP-1 human monocytic leukemia cells with combinations of DCA or CDCA with ATRA was also beneficial in inducing monocytic differentiation. These cells are known to respond only to very high doses of ATRA (21). However, when combined with bile acids, the dose of ATRA can be considerably lowered (~10-fold).

Effect of PKC Inhibitors on Cell Differentiation Induced by Bile Acids. We have shown previously that the PKC inhibitor sphinganine inhibited the differentiation induced by the bile acids in HL60 cells (5), thus implying an important role of PKC as a mediator in this process. This is in agreement with previous reports obtained with purified rat brain PKC, in rat and human colon, and cell lines treated with bile acids (15–17). Here, we have tested another PKC inhibitor, H-7, at 10 and 20 μM (22). Results presented in Fig. 5 show that 20 μM H-7 inhibited significantly the cell differentiation induced by the three bile acids. A reduced inhibition level was obtained with 10 μM H-7 (data not shown). Interestingly, the inhibition of HL60 cell proliferation by bile acids was not much attenuated by H-7. When administered alone, 10 or 20 μM H7 had no effect on cellular differentiation and inhibited cell proliferation by 34% only at the higher concentration of 20 μM (data not shown).

Because staurosporine is a very potent protein kinase inhibitor that enhances or induces differentiation in various cell types (23, 24), we tested this drug in combination with bile acids. At 5 nM, staurosporine...
alone did not significantly affect HL60 cell proliferation and differentiation. However, when combined with bile acids, it strongly enhanced cellular differentiation (Fig. 5). The potentiation of the effect of bile acids by 5 nM staurosporine was also associated with a markedly enhanced antiproliferative effect and only slight cytotoxicity. At a higher concentration of 20 nM, staurosporine alone induced considerable cell differentiation (61% NBT-positive HL60 cells), and when combined with bile acids it enhanced further their effect. However, these treatments were cytotoxic, resulting in 60–75% cell viability (data not shown).

Morphological examination of maturation and differentiation of HL60 cells treated with H-7 or staurosporine, alone and in combination with bile acids, agreed with results obtained in NBT test. Also, there was an additive effect of these drug combinations on the morphological appearance of apoptotic cells, which increased respectively from 3 to 8% with 5 nM staurosporine and bile acids alone to 14–18% in the combination treatments.

Effect of Bile Acids and Their Combination with ATRA or Vit D₃ on Mbn Gene Expression. The Mbn gene encodes a serine protease (leukocyte proteinase 3) and is specifically expressed in human promyelocytic hematopoietic cells (25). It is down-regulated during both normal myelopoiesis and the differentiation of human promyelocytic cell lines, including HL60 cells treated with different inducers, such as ATRA, Vit D₃, and PMA (11). Inhibition of Mbn expression by specific antisense oligodeoxynucleotide was shown to cause monocytic differentiation of HL60 cells (11). We were, therefore, interested to test the effect of bile acids on the expression of Mbn transcripts in HL60 cells. We first examined the effect of CDCA (75 μM) and LCA (60 μM) in concentrations that were shown to maximally induce differentiation in HL60 cells (5). These conditions were compared with 0.5 μM ATRA and 50 nM Vit D₃.

Results presented in Fig. 6A show that Mbn mRNA levels (1.3 kb) were remarkably decreased in cells treated for 5 days with the bile acids LCA (95% decrease) and CDCA (90%), and a similar effect was observed with the differentiation-inducing agents ATRA or Vit D₃ (95%), in agreement with previous results (11). Densitometric analysis of the Mbn transcripts normalized with the 28S RNA bands showed that addition of 0.1 and 1 μM ATRA decreased the expression of Mbn by 15–25% at day 2 and 25–80% at day 4, respectively (data not shown). In comparison, a time course study with LCA (60 μM) in Fig. 6B showed that this bile acid down-regulated Mbn transcripts slightly at day 1 (10% decrease) but very significantly at day 2 (40%). This effect was still evident and persistent at day 5 (65%). In addition, this down-regulation of the Mbn message by LCA at day 2 preceded
the effect of this bile acid on the NBT test, which occurred only at days 3–4 (5). This is significant, because we observed that the NBT reaction (oxidative burst) is typically preceding the cell cycle arrest and the morphological changes that accompany HL60 cell maturation and differentiation (5, 26).

We then studied the effect of a combination of a low concentration of CDCA (50 μM, which alone did not induce differentiation in HL60 cells), together with low doses of ATRA (2 nm) or Vit D₃ (5 nm). Fig. 6C shows that the combination of CDCA with Vit D₃ caused additional down-regulation in Mbn mRNA (90%), as compared with the change observed by either of these drugs: 65% with CDCA or 45% with Vit D₃. In contrast, addition of 2 nm ATRA, inducing 31% decrease in Mbn transcript, with 50 μM CDCA (65% decrease) were not additive (35% decrease).

As mentioned above, down-regulation of the Mbn gene precedes the differentiation of HL60 cells triggered by different inducers and is not related to the “decision” to follow the monocytic or the granulocytic pathways of maturation-differentiation (11). The fact that bile acids alone, or in combination with another effective inducer, can down-regulate Mbn is interesting; and the specific combination of CDCA plus Vit D₃ may be of special relevance because both drugs are already used clinically (although for different objectives). The combination of CDCA with ATRA was not additive in terms of Mbn...
mRNA expression (Fig. 6C), in spite of the beneficial effect of this combination in inducing HL60 cell differentiation (shown by NBT test and morphological examination). However, posttranscriptional and/or posttranslational effects are certainly possible.

DISCUSSION

In this study, we have demonstrated that bile acids, compounds that were shown previously by us to induce differentiation of HL60 cells in vitro (5), cooperate with ATRA or Vit D₃ to induce a more pronounced differentiation phenotype in human leukemic cells HL60 and THP-1. The concentrations of these two morphogens could be lowered about 5–10 times, to levels that by themselves had no effect or were only slightly effective. Because ATRA and Vit D₃ exert side effects when used clinically, lowering their concentration without losing therapeutic gain is important. These drug combinations induced mainly monocytic cell differentiation. However, it appeared that under certain drug combinations, a mixed population of two lineages could emerge, similar to previous findings (20). In our studies (5) and herein, we have shown that both primary (CDCA) and secondary (DCA and LCA) hydrophobic bile acids can inhibit HL60 cell proliferation and induce maturation and differentiation. Cholic acid and ursodeoxycholic (50 to 200 μM), which are water soluble, did not have such effects and even stimulated cell proliferation (data not shown). Indeed, bile acids can also enhance cellular proliferation, in vitro and in vivo, independent of cytotoxicity (27). In HT-29 human colon cancer cells, this effect was confined to narrow concentration “windows,” which differed for the different bile acids (28), similar to the situation we described.

The cooperative effect of bile acids with Vit D₃ or ATRA may be explained as follows. Bile acids were shown previously to affect PKC activity (15–17), to enhance the phosphorylation of selected PKC substrates (15), and to exert a differential effect on PKC isoforms (29). Recently, deoxycholate was found to increase the expression of c-fos mRNA and fos/jun binding to API sites in HT-29 human colon cancer cells (30). Also, Hirano et al. (31) have shown that CDCA specifically induced API binding activity in nuclear extracts of human colon carcinoma Lovo cells.

Our results obtained here with H-7 revealed that PKC is an important mediator of the effect of bile acids on HL60 cellular differentiation. In agreement, PKC isoform (32–34) and API response elements (35, 36) were strongly implicated in the regulation of proliferation and monocytes/macrophage differentiation of human leukemic cells. The divergent effects of the PKC inhibitors H-7 and staurosporine on differentiation might be explained by their differential activity on the PKC isoforms. In addition, not all cellular targets of staurosporine are known yet, and variable effects of this drug on cell cycle arrest and apoptosis were described recently (37–41). Staurosporine administered at 10–200 nm was shown to induce apoptotic bodies, without concomitant DNA fragmentation in MOLT-4 cells (40). In HL60 cells, it caused DNA fragmentation only at concentrations 10–40 times higher than those used herein (41). In our experiments, the combination of staurosporine with bile acids (DCA and LCA) increased the number of morphologically apoptotic HL60 cells in an additive manner. It might be useful, therefore, to explore further the mechanism related to positive interactions between bile acids and staurosporine implicated from our data.

Another important observation reported herein is the down-regulation of the Mbn gene induced by bile acids in HL60 cells. Mbn is a neutral serine proteinase, identical to proteinase 3 (25). Mbn gene expression is confined to the promyelocytic stage of polymorphonuclear leukocyte maturation and is switched off upon myeloid differentiation. Little is known about its biological targets. However, Mbn was recently shown to hydrolyze specifically the M₈ 28,000 heat shock protein hsp 28 involved in cellular growth and differentiation (42). Mbn is a common target to a variety of inducers of promyelocytic cellular differentiation, independent of the choice between monocytic versus granulocytic pathways (11, 43).

Our data therefore strongly suggest that the Mbn promoter is a possible target of bile acids or transcription factors under the control of signaling pathways activated by bile acids and PKC-dependent mechanisms. In favor of this hypothesis is the recently described mechanism for the control of liver cholesterol 7α-hydroxylase gene (CYP7α). This gene is regulated by a negative feedback of hydrophobic bile acids reaching the liver by enterohepatic circulation and also by cholesterol and by steroid and thyroid hormones. BARE-I and BARE-II were identified in the CYP7α promoter using DNase I footprinting, electrophoretic mobility shift assay and transient transfection of HepG2 cells with a promoter/luciferase reporter gene (44, 45). BARE-I was shown to contain a direct repeat of hormone response elements separated by four nucleotides (DR4). Chicken ovalbumin upstream promoter transcription factor (COUP-TFII) binds this DR4 and transactivates the CYP7α gene. DR4 also binds the liver X receptor/RXR complex mediating oxysterol-dependent transactivation (44, 45). BARE-II is the major BARE element involved in the transcriptional repression of CYP7α by hydrophobic bile acids. It also contains three HRE-like sequences that form two overlapping nuclear receptor binding sites. One is a direct repeat separated by one nucleotide DR1 (-146-TGGACTTAGTTCA-134), and the other is a direct repeat separated by five nucleotides DR5 (-139-AGTTCA aggcc GGGTAA-123). The DR5 binds the RXR/retinoic acid receptor heterodimer.

BARE-I and BARE-II are highly conserved in different mammalian species and share a novel sequence, AGTCCAG. Most interestingly, the Mbn promoter (46, 47) also harbor a BARE-II like sequence (AGTCCAG) between nucleotides −156/−143 and several components of the transcription machinery identified in the CYP7α promoter, including Vit D₃ and thyroid hormone response elements, and particularly, the RXR/retinoic acid receptor motif overlapping BARE-II. Very recent data identified a bile acid signaling pathway leading to the activation of the orphan nuclear receptor farnesoid X receptor, thereby regulating the expression of CYP7α and the intestinal bile acid binding protein I-BABP (48–50). The I-BABP promoter is activated by the farnesoid X receptor and bile acids and contains the inverted repeat response element (IR-1; Ref. 49) that functions as a palindromic BARE (AGGTGAATTTCA). More details of these molecular interactions are needed to clarify the role of the BARE-like sequence in the Mbn promoter using reporter gene assays and electrophoretic mobility shift assay. Posttranscriptional modulations might also occur (25). All of these possibilities need further investigation.

Until recently, bile acids were considered in the context of human cancer mostly as harmful tumor-promoting agents. However, a closer examination of their physiological roles and biochemical relationships to cholesterol and its steroid hormones and Vit D₃ metabolites, and considering the convergence in their interactions with nuclear proteins/receptors and genomic response elements, bile acids make attractive candidates for useful purposes. Some bile acids are already used in therapeutics (51, 52). Their chemical modifications might also improve their benefits in the future (53). Taken together, our data suggest that bile acids and their derivatives may exert a therapeutic effect in human leukemic cells. These compounds may also have important functions in normal development and in the neoplastic progression of the gastrointestinal mucosa.
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