Novel Target Genes of the Ah (Dioxin) Receptor: Transcriptional Induction of N-Myristoyltransferase 2

Siva Kumar Kolluri, Carolin Balduf, Martin Hofmann, and Martin Göttlicher

Forschungszentrum Karlsruhe, Institute of Toxicology and Genetics, D-76021 Karlsruhe, Germany

ABSTRACT

Dioxins are potent mammalian carcinogens and toxins affecting skin, liver, and immune and reproductive systems. The intracellular Ah receptor, a ligand-dependent transcription factor of the basic region/helix-loop-helix/Per-Ahr/Arnt-Sim homology domain (bHLH-PAS) protein family, mediates responses to dioxins. Target genes of the Ah receptor that mediate dioxin toxicity and carcinogenicity are, however, mostly unknown. We used 5L rat hepatoma cells to identify dioxin-inducible genes by suppression subtractive hybridization. Eleven cDNA fragments were identified that represented novel sequences or genes for which induction by dioxins had not been known. N-myristoyltransferase 2 (NMT2) is one of the later dioxin-inducible genes. Induction of NMT2 was confirmed in livers of mice in vivo. NMT2 induction was a direct consequence of Ah receptor activation in 5L cells. [3H]myristic acid incorporation into 5L cell proteins was inducible by dioxins, indicating that protein myristoylation is a regulated rather than a housekeeping function and that NMT activity is limiting in noninduced 5L cells. Here we show for the first time that expression of NMT2 and induced protein myristoyltransferase activity are direct responses to carcinogen exposure. Because inappropriate protein NH2-terminal myristoylation appears to play a role in carcinogenesis, induction of NMT2 may play a central role in dioxin carcinogenicity.

INTRODUCTION

Dioxins like TCDD evoke a broad spectrum of biochemical and toxic effects in a variety of species including immune suppression, skin alterations, sperm count reduction, body weight loss, and carcinogenicity in the rodent liver as well as in human (1, 2). Dioxin effects are mediated by the AhR, which is a member of the basic region/helix-loop-helix/Per-Ahr/Arnt-Sim homology domain (bHLH-PAS) family of transcription factors (3, 4). The AhR functions as a ligand-activated transcription factor. Binding of dioxins to AhR triggers formation of dimeric complexes with the partner protein, Arnt. The AhR/Arnt heterodimer binds to recognition sites in the promoters of target genes and enhances their transcription. Known target genes that have served as model cases for the analysis of AhR function mostly comprise genes involved in xenobiotica metabolism, such as members of the cytochrome P-450 subfamily and enzymes of phase II metabolism (5–7). Genetic evidence in mice (8–10) indicate that most, if not all, activities of dioxins are mediated by the AhR, but there are indications that not all of these activities may require Arnt (11, 12).

Despite the recognition that dioxins exert their biological functions by binding to AhR, mechanisms of dioxin toxicity and carcinogenicity are not well understood because only a few target genes other than those related to xenobiotica metabolism are known. For those target genes that are known, the relevance for dioxin toxicity and carcinogenicity is unclear. Recently, we reported that AhR directly up-regulates the expression of the cell cycle inhibitor Kip1 and, by that, inhibits cell proliferation both in 5L hepatoma cells and in fetal thymus glands (12). However, AhR target genes that mediate other aspects of toxicity and carcinogenicity in particular are poorly understood. It is likely that the genetic responses to AhR activation are complex and that, in addition to the known dioxin-regulated genes, there are other target genes of AhR that ultimately support tumorigenic transformation of cells in the presence of dioxins. Cell culture models in vitro may be of limited value in the study of the process of carcinogenesis as a whole but may provide useful models to identify target genes immediately downstream of the carcinogen-activated AhR.

Several attempts have been made to systematically identify changes in gene expression after TCDD exposure by differential RNA expression screening approaches. Sutter et al. cloned several TCDD-inducible cDNAs, including those for tissue plasminogen activator inhibitor 2 and interleukin 1β (13). Gao et al. found ecto-ATPase induced by dioxins (14). Selmin et al. as well as Donat and Abel reported (15, 16) induction of several genes using the differential display technique. Induction of these genes provides evidence that TCDD alters expression of many genes in different cells/organs, but obvious explanations for most of the toxic activities of dioxins and tumor promotion in particular have not been found.

Thus, there is considerable interest in identifying AhR-regulated genes by technologies that are suitable for detecting all or at least most of the dioxin-induced alterations in mRNA expression, including those concerning low-abundance mRNA species. In this study, we used the dioxin-responsive 5L hepatoma cell line (17–20) to identify AhR target genes by SSH (21, 22). SSH was used to take advantage of a normalization step increasing the relative representation of low-abundance mRNAs; and SSH was preferred over screening of cDNA arrays to allow identification of novel genes. Six known TCDD-inducible genes were found together with 11 novel cDNA fragments. We show that NMT2 is one of the genes induced by TCDD in 5L cells and in the mouse. At least in 5L cells, the induction was direct and strictly dependent on AhR. The increase in NMT2 mRNA lead to increased protein myristoylation in pulse-labeled 5L cells, providing the first example of protein myristoylation being susceptible to short-term regulation by a chemical carcinogen.

MATERIALS AND METHODS

Cell Culture. Cells were maintained in DMEM supplemented with 10% fetal bovine serum as described previously (12); TCDD was added as 1 μM stock solution in DMSO to a final concentration of 1 μM and control cells were treated with 0.1% DMSO.

Northern and Southern Blot Analyses. Northern blot analysis was done using 5 μg of poly(A)+ RNA. Probes were prepared by PCR or restriction digestion from individual clones as described previously (21, 22). cDNA samples (10 μg each) were labeled in random prime reactions (Amersham) with [32P]dCTP (3000 Ci/mmol), and probes were purified by size exclusion.
chromatography (Nick columns, Pharmacia Biotech). Incorporation of radioactivity was determined by liquid scintillation counting. Only probes that had incorporated between 15 and 30 μCi of 32P were used for hybridization reactions. RNA loading was controlled by hybridization with a GAPDH probe. Southern blot analysis was done as described previously (23).

Generation of a Subtracted cDNA Library by SSH. SSH was performed between nontreated 5L cells (driver) and 4-h-TCDD-treated 5L cells (tester), using the PCR-Select cDNA Subtraction kit (Clontech Laboratories, Inc.). All of the PCR and hybridization steps were performed on a Perkin-Elmer 9600 thermal cycler. Double-stranded cDNA was synthesized from 2 μg of poly(A) RNA of the non-treated or the 4-h-TCDD-treated cells as described previously (23). Both double-stranded cDNAs were then RsaI-digested, precipitated, and redissolved in freshly autoclaved water. Tester cDNA was ligated with adaptors (21). The ligation efficiency was verified by comparing PCR products obtained with gene-specific primers for GAPDH or one GAPDH-specific primer and another adaptor-specific primer. The difference between the intensities of the above PCR reactions was less than 2-fold. For the first hybridization the mixture of driver and tester cDNAs was denatured at 98°C for 90 s and then incubated at 68°C for 11 h. For the second hybridization, fresh driver cDNA was denatured at 98°C for 90 s; then the samples from the first hybridization were mixed together without denaturing in the presence of freshly denatured driver cDNA and were incubated at 68°C for 20 h. At the end of the incubation period, 200 μl of dilution buffer [20 mm HEPES-HCl (pH 8.3), 50 mM NaCl and 0.2 mM EDTA] were added. Differentially expressed cDNAs were selectively amplified in two rounds of PCR as described previously (21).

Colony PCR. To identify TCDD-induced genes, individual clones from the subtractive library were picked, and the inserts were amplified by colony PCR. DNA was denatured with alkali treatment, and duplicate sets of dot blots were prepared in 96-well-format. These dot blots were then hybridized with radioactively labeled double-stranded cDNA derived from either nontreated or 4-h-TCDD-treated 5L cells.

RACE. A commercially available adaptor ligated double-stranded cDNA (Marathon-Ready cDNA from Clontech Laboratories, Inc.) was used as a template. PCR-amplification of 3’ and 5’ regions, extending the novel clone 106, was performed using one adapter and one clone 106-specific primer, each in separate PCR reactions. Primers used were 5’-gcacccagctggtcagttgccct and 5’-gaccagccggtgtatgtctgc. Products were subcloned, sequenced, and compared with the database.

RT-PCR Analysis of Mouse Liver Samples. RT-PCR analysis was performed using first-strand cDNA reversibly transcribed from 0.1 μg of total liver RNA by oligo(T) priming and NMT2-specific primers (clone 106 primers, see above) and GAPDH-specific primers, 5’-accaagcatcgccatcaca-3’ and 5’-tccacctgggtgtgtaccc-3’ in a single-tube hot-start PCR reaction with the following conditions: 25 cycles for 30 s at 94°C, 45 s at 58°C, and 90 s at 72°C. Pilot experiments had confirmed that the PCR amplification of both NMT2 and GAPDH was linear. Five μl of the PCR products were separated on an agarose gel, and Southern blot analysis was performed with radiolabeled GAPDH- and NMT2-specific probes. A phosphorimager (FUJI BAS 2000) was used for quantitative analysis.

In Vivo Labeling of Cells. 5L cells were treated with 1 μM TCDD or 0.1% DMSO solvent for 16 h or 48 h and were then incubated with [3H]myristic acid (50 μCi/ml) for an additional 3, 5, 8, or 12 h. Cell extracts were prepared in SDS-PAGE sample buffer, and proteins were separated by SDS-PAGE, together with radioactively labeled and prestained size markers (Amersham). Autoradiography was performed with the Amplify fluorographic reagent (Amersham Buchler, Braunschweig, Germany). Extracts for immune precipitations were prepared in standard RIPA buffer (PBS, 7% NP40, 0.5% sodium deoxycholate, and 0.1% SDS). Antibodies against src were Ab-1 (Calbiochem) or sc-18 (Santa Cruz Biotechnology). Against src-like kinases, we used: Blk (sc-329); Fgr (sc-17); Hck (sc-1428); Lck (sc-433); Lyn (sc-7274; all from Santa Cruz Biotechnology).

RESULTS

Generation of a cDNA Library Enriched for TCDD-induced Genes. Poly(A)+ RNA from nontreated and 4-h-TCDD-treated 5L cells was used for generation of a subtractive cDNA library (TCDD-treated minus nontreated) using the PCR-Select cDNA subtraction method (21, 22). This procedure also normalizes between low and highly abundantly expressed mRNAs allowing for a reasonable probability to also recover low-abundance transcripts from the generated library. Northern hybridizations confirmed the presence and inducibility of known TCDD-induced transcripts such as cytochrome P450 1A1 (CYP450 1A1) and glutathione-S-transferase Ya (GST-Ya) in the mRNA preparations used for library generation (data not shown). Subtraction efficiency was determined by monitoring depletion of cDNAs common to both induced and noninduced populations and enrichment of sequences specific to the induced population. Southern blot analysis of subtracted and unsubtracted cDNA pools showed that one abundant nondifferentially expressed gene, GAPDH, was almost completely removed. The cDNAs of CYP450 1A1 and GST-Ya, two known TCDD-induced genes, were enriched in the subtracted cDNA population (Fig. 1). This indicated the efficient generation of a pool of cDNA fragments enriched for those fragments derived from TCDD-induced genes. The pool was cloned as plasmid library.

Identification of Individual TCDD-induced Genes in the Subtractive Library. A total of 1500 clones of the library were picked for further analysis by colony PCR amplification of the library inserts and dot blot hybridization. A representative set of hybridizations is shown in Fig. 2. Replica filters of 96 PCR fragments, together with positive (TCDD-inducible CYP450 1A1) and negative (non TCDD-inducible GAPDH) controls, were hybridized with radiolabeled cDNA derived from RNA of either control cells (upper) or cells that had been treated with TCDD for 4 h (lower). Of the 1500 clones picked, 34 isolates preferentially hybridized with radiolabeled cDNA derived from TCDD-treated cells. Identity of cDNA clones that hybridized preferentially with radiolabeled cDNA from TCDD-treated cells was determined by plasmid DNA isolation and sequence analysis. The 34 cDNA fragments belonged to 17 apparently independent clusters (Table 1), which included 6 known dioxin-inducible genes, e.g., CYP450 1A1 (4 isolates), CYP450 1B1, aldehyde dehydrogenase (13 isolates), GST-Ya, NAD(P)H:quinone oxidoreductase and UDP-glucuronosyl transferase (2 isolates).

Besides the known TCDD-inducible genes, six genes were identified that were hitherto not known to be controlled by dioxins. These were B-cell translocation gene 1 (BTK-1; Ref. 24), lost on transformation 1 (LOT-1; Ref. 25), transglutaminase (26), a cytokine receptor-related protein (Cyto R4; Genebank accession no. AF046060), an ABC transporter (ABC1; Ref. 27), and a cDNA that is overexpressed...
in N-methyl-N-nitrosourea-induced rat mammary tumors (T4 clone; Ref. 28).

One isolate, clone 1396, showed extensive homology to ESTs of the mouse (GenBank accession nos. A1451155, B686290, Aw122414, and Be648297). These four ESTs also showed homologies to each other so that a putative contiguous sequence of 1023 bp could be assembled. The sequence is partial, however, because Northern blot hybridization suggested that the endogenous mRNA is ~7.5 kb in size. Four isolates did not match any cDNA database entry (Table 1). These cDNA clones may represent genuine novel genes or may correspond to hitherto not published regions of known genes.

TCDD-inducibility of newly identified genes was confirmed by Northern blot analysis (Fig. 3). In three cases (clone 735, lot1 and cyto R4) the probe identified two bands of which, in the cases of clone 735 and cyto R4, only one was inducible by TCDD. These clones may represent two closely related genes out of which only one is induced by TCDD. Alternatively, the double bands on Northern blots may result from an artifact due to generation of the subtractive library if fragments from two cDNAs were ligated into one plasmid, although there is currently no evidence from sequence analysis to support this possibility. In all other cases, a single band and bona fide inducibility by TCDD was confirmed.

Furthermore, the Northern blot analysis showed that cDNA fragments had been cloned from both minimally and highly expressed genes. Signal intensity in Northern hybridization analysis as well as the inducibility of the novel genes by TCDD differed considerably (Fig. 3). Some clones were induced more than 10-fold (clones 1531, 735, and CytoR4) and others only <4-fold (BTG1, LOT1, transglutaminase, ABC1, and clones 106 and 1396). Thus, the chosen experimental approach was suitable to obtain clones corresponding to genes that are not heavily regulated or that are expressed at a low level.

Table 1 Database queries on TCDD-induced cDNA clones

<table>
<thead>
<tr>
<th>Annotation of cDNA</th>
<th>No. of clones</th>
<th>Accession nos.*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytochrome P450 1A1</td>
<td>4</td>
<td>M26129</td>
</tr>
<tr>
<td>Cytochrome P450 1B1</td>
<td>1</td>
<td>U09540</td>
</tr>
<tr>
<td>Aldehyde dehydrogenase</td>
<td>13</td>
<td>J03657</td>
</tr>
<tr>
<td>Glutathione-S-transferase Ya</td>
<td>1</td>
<td>M14986</td>
</tr>
<tr>
<td>NAD(P)/H-quinone reductase</td>
<td>1</td>
<td>M31805</td>
</tr>
<tr>
<td>UDP-glucuronosyl transferase</td>
<td>1</td>
<td>U75903</td>
</tr>
<tr>
<td>Group II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B-cell translocation gene 1</td>
<td>1</td>
<td>L26268</td>
</tr>
<tr>
<td>Lost on transformation 1</td>
<td>1</td>
<td>U72626</td>
</tr>
<tr>
<td>Transglutaminase</td>
<td>1</td>
<td>M55154</td>
</tr>
<tr>
<td>Cytokine receptor-related protein 4</td>
<td>1</td>
<td>AF0486060, AF120152</td>
</tr>
<tr>
<td>T4 clone</td>
<td>2</td>
<td>U30788</td>
</tr>
<tr>
<td>ABC1</td>
<td>1</td>
<td>X75926</td>
</tr>
<tr>
<td>Group III</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clone 106 (= NMT2, see text)</td>
<td>1</td>
<td>AF043327 (by Race, see “Results”)</td>
</tr>
<tr>
<td>Clone 361</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Clone 735</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Clone 1396</td>
<td>1</td>
<td>Cluster of ESTs (see “Results”)</td>
</tr>
<tr>
<td>Clone 1531</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

* The sequence of cDNA clones was compared with the Genbank, EMBL, and EST databases by BLAST analysis (41). If high homologies were found, cDNA annotations and accession numbers are shown.

expression is identical to that seen with clone 106 (data not shown). Thus, clone 106 corresponds to a yet unknown fragment of the 3' untranslated region of rat NMT2 (Fig. 4; Ref. 29).

**AHR-dependent Induction of NMT2 Transcription by TCDD.** To test whether the induction of NMT2 strictly depends on the AhR, subclones of 5L cells (17, 20) were analyzed. NMT2 was not inducible by TCDD in the AhR-deficient BP8 subclone, which lacks AhR expression (Fig. 5A). The induction was restored by stable expression of AhR in these BP8 cells, which indicated that AhR is required for NMT2 induction.

NMT2 mRNA was not inducible by TCDD in 5L cells pretreated with the transcriptional inhibitor actinomycin D, which suggests that TCDD-induced NMT2 mRNA levels are attributable to increased transcription (Fig. 5B). Nuclear run-off analysis provided direct evidence for the induction of NMT2 mRNA synthesis rate by TCDD (Fig. 5C). NMT2 could be a direct target gene of AhR or could require the synthesis of intermediary proteins that induce NMT2. To discriminate between the possibilities, TCDD-inducibility of NMT2 mRNA was tested in the presence of the protein synthesis inhibitor cycloheximide (Fig. 5D) under conditions that had been shown previously to efficiently block protein synthesis in 5L cells (12). TCDD induced NMT2 mRNA in both the absence and the presence of cycloheximide. Thus, ongoing or *de novo* protein synthesis are not required for induction of NMT2 mRNA by TCDD, which indicates that NMT2 is directly regulated by AhR. This is consistent with the early occurrence of mRNA induction in a time-course analysis (Fig. 5E).

**In Vivo Induction of NMT2 by TCDD.** A prerequisite for any target gene of AhR to mediate dioxin toxicity or carcinogenicity is expression in the relevant target organ. TCDD is a potent promoter of carcinogenesis in the rodent liver (30). Thus, the inducibility of NMT2 mRNA in livers of TCDD-treated mice was tested. RT-PCR followed by Southern blotting analysis of the specific product revealed NMT2 mRNA up-regulation in the liver of mice after 3 (2.5 fold), 5 (2.5 fold), or 7 days (4 fold) of TCDD treatment (Fig. 5F). PCR amplification could be expected to be in the linear range because much higher signals, but similar ratios between samples, were obtained when the PCR was continued for five additional cycles (data not shown). Furthermore, at the chosen number of cycles, the product was not yet detectable by ethidium bromide staining of agarose gels.

**Dioxin-induced Increase in Protein Myristoylation.** 5L cells were labeled *in vivo* with [3H]myristic acid to test whether induction of NMT2 by TCDD is functionally relevant, i.e., would lead to increased myristoylation of cellular proteins. Autoradiography of PAGE-separated whole cell extracts indicated that TCDD pretreatment specifically induced the occurrence of a prominent radiolabeled band at the size of M_r ~ 55,000 (Fig. 6A). Minor inducible bands occurred at M_r ~ 80,000 and 100,000, whereas the abundance of other radiolabeled bands, e.g., at M_r 40,000, did not depend on TCDD pretreatment. This indicated that in the noninduced cells NMT-2 activity is limiting with respect to several substrate proteins and that TCDD treatment can indeed induce myristoylation of specific target proteins. The c-Src tyrosine kinase is a known myristoylated protein, but the prominent protein is smaller than c-Src (Fig. 6A). It comigrates, however, with a protein that is also recognized by one of the antibodies directed against c-Src and, therefore, may correspond to another member of the Src kinase family. In 5L cell extracts, antibodies against known family members detect Blk, Lyn, and low levels of Fgr, in addition to c-Src. Lyn and Blk can be expected to migrate at the position of the inducibly myristoylated protein. In the case of Lyn, a suitable antibody was available for immune precipitation of [3H]myristoylated protein from 5L cell extracts (Fig. 6B, right lanes). The experiment proves that the chosen approach is suitable for de-
tecting myristoylation of specific proteins such as Src-like kinases. However, Lyn myristoylation seems not to depend on TCDD treatment of 5L cells, because labeling of precipitated Lyn is not induced, and Lyn migrates slightly more slowly than the predominant inducibly myristoylated protein (Fig. 6B). Thus, at present, the identity of the inducibly myristoylated protein remains uncertain, and purification for mass spectroscopic identification will be required.

**DISCUSSION**

The AhR mediates toxic and carcinogenic effects of dioxins. Known AhR-regulated genes mostly code for xenobiotica-metabolizing enzymes, but AhR-regulated genes responsible for dioxin toxicity were largely unknown. Those genes that do not code for xenobiotica-metabolizing enzymes do not conclusively explain all aspects of dioxin toxicity or carcinogenicity. Thus, we used SSH to identify novel target genes of AhR. The major outcome of this search were the identification of 11 unexpected or novel cDNA fragments and, most importantly, the finding that NMT2 expression and protein myristoylation underly short-term regulation, e.g., by TCDD.

SSH was chosen instead of a CDNA array-based analysis for several reasons; first, array-hybridization usually does not involve a normalization step, which precludes detection of low-abundance cDNAs.

Second, the labeling reaction in common protocols prefers the 3′-end of RNAs and thus limits the analysis to cDNAs and ESTs corresponding to 3′-ends of known cDNAs. Finally, we wanted to allow identification of novel sequences. The analysis of the obtained results indicates that, both, the identification of low-abundantly expressed genes as well as the isolation of novel sequences was successful. Seven cDNA clones including the RACE-extended clone of NMT2 correspond to known genes for which inducibility by TCDD is novel. Four cDNA fragments represent novel sequences. Some of the known TCDD-induced RNAs were represented by up to 13 different cDNA clones, whereas other RNAs were represented by only one cDNA clone. Thus, a major fraction of the TCDD-induced alterations in the transcriptome appears to be covered, but complete saturation could not be assured despite picking a moderately high number of clones.

This study indicates an estimate of the complexity of AhR-induced early changes in gene expression because several unexpected genes were found. Increased transglutaminase enzyme activity has been reported in the epidermis of hairless mice by topical treatment with TCDD (31) and epidermal cells treated in vitro (32). The present study might explain these former findings by showing early induction of transglutaminase mRNA by TCDD. Among the other known genes induced by TCDD, BTG1 is reported to negatively regulate proliferation (24). LOT1 is a novel gene cloned based on its loss of expression in malignant transformed cell lines (25). T4 clone, a gene containing retroviral sequences, is up-regulated in rat mammary tumors induced by N-methyl-N-nitrosourea (28). In addition, TCDD also induced ABC1, which codes for a member of the large transmembrane ATP-binding cassette protein family. These proteins transport diverse substrates, such as sugars, amino acids, proteins, metals, and xenobiotica across membranes (27). Recently, it is reported that ABC1 is transcriptionally regulated by sterols through nuclear receptors liver-X-receptor (LXR) and retinoid-X-receptor (RXR; Ref. 33). ABC1 induction by AhR might, much like xenobiotica-metabolizing enzymes, play a role in the organism’s machinery to locate xenobiotica to certain compartments and to eliminate them. The functional and possibly pathophysiological roles of other induced genes, such as BTG1, LOT1, CYTO B4, and T4 clone, are currently not known.

The most prominent result of this study is the finding that NMT2 expression is inducible by a small diffusible compound, i.e., the xenobiotic TCDD, by a receptor-dependent mechanism. NMT2 is an enzyme-catalyzing protein N-myristoylation, i.e., the cotranslational linkage of myristic acid to the NH2-terminal glycine residues of eukaryotic cellular proteins (34). Examples of NMT substrates include protein tyrosine kinases like p60src, cAMP-dependent protein kinase (PKA), the Gs G-protein subunit and the calcineurin B protein phosphatase. Human and mouse cDNAs for two distinct NMTs called NMT1 and NMT2 have been cloned. The specific role of each NMT in protein myristoylation has not yet been studied (29). Furthermore, there are no published data on the transcriptional regulation of NMTs. The AhR constitutes the first conditionally regulated pathway that controls expression of either of the NMT genes.

NMT2 mRNA inducibility raises the questions as to whether increased NMT2 expression translates into increased protein myristoylation and whether the induction occurs in the living animal. Increased protein myristoylation could be shown in 5L cells because dioxin pretreatment induced increased appearance of myristoylated proteins. Furthermore, AhR-dependent regulation of NMT2 appears to be relevant also in the mouse, because NMT2 mRNA was increased in livers of mice after 3, 5, or 7 days of TCDD treatment. Thus, NMT2 fulfills essential requirements to be a candidate gene in mediating toxic or carcinogenic properties of dioxins, e.g., its activity is limiting in the absence of the inducing agent, and the induction by TCDD occurs in a target organ of dioxin action.
A causal role of NMTs in carcinogenesis is suggested by the finding of elevated levels of NMT protein and NMT activity in carcinomas of the colon (35, 36) or the gallbladder (37). The mechanism(s) of up-regulation are currently not known. One potential myristoylated target protein might be c-src, because p60-src potently induces cell transformation (38), and inhibition of protein myristoylation also prevents transformation (39, 40). Thus, inappropriate protein myristoylation is associated with several forms of cancer and is likely to play a role in pathogenesis, although some of the relevant myristoylated proteins may not yet be known. Because TCDD induces NMT2 in vivo, it is tempting to speculate that ectopic NMT2 expression mediates carcinogenicity of TCDD by induction of inappropriate protein myristoylation.

ACKNOWLEDGMENTS

We thank Dr. von Stein for helpful methodological suggestions, Peter Herrlich, Andrew Cato, Erik Wade, and Carsten Weiss for stimulating discussions. Albrecht Buchmann and Michael Schwarz from the University of Tübingen, Tübingen, Germany, generously provided liver samples from TCDD-treated mice. Sebastian Maurer-Stroh, Research Institute of Molecular Pathology, Vienna, was a valuable partner in discussing protein myristoylation. Anke Pelzer and Elke Martin are gratefully acknowledged for excellent technical support.

REFERENCES

10. Dhahbi, J. M., Sutter, T., Bell, D. Isolation and characterization of a novel gene induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin in rat liver. Carcinogenesis (Lond.), 17: 2609–2615, 1996.
12. Göttilicher, M., Cikryt, P., and Wiebel, F. J. Inhibition of growth by 2,3,7,8-tetrachlorodibenzo-p-dioxin in 5L rat hepatoma cells is associated with the presence of Ah receptor. Carcinogenesis (Lond.), 11: 2205–2210, 1990.
Novel Target Genes of the Ah (Dioxin) Receptor: Transcriptional Induction of N-Myristoyltransferase 2

Siva Kumar Kolluri, Carolin Balduf, Martin Hofmann, et al.

Cancer Res 2001;61:8534-8539.

Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/61/23/8534

This article cites 40 articles, 13 of which you can access for free at:
http://cancerres.aacrjournals.org/content/61/23/8534.full#ref-list-1

This article has been cited by 4 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/61/23/8534.full#related-urls

Sign up to receive free email-alerts related to this article or journal.
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.