0026-895X/99/040668-09
Copyright © 1999 by U.S. Government
All rights of reproduction in any form reserved.
MOLECULAR PHARMACOLOGY, **55:**668–676 (1999).

MOL PHARM

Post-Transcriptional Regulation of *MyD118* and *GADD45* in Human Lung Carcinoma Cells During 6-[3-(1-Adamantyl)-4-hydroxyphenyl]-2-naphthalene Carboxylic Acid-Induced Apoptosis

MORITO SAKAUE, HIROSHI ADACHI,[1] and ANTON M. JETTEN

Cell Biology Section, Laboratory of Pulmonary Pathobiology, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, North Carolina

Received October 19, 1998; accepted January 7, 1999

This paper is available online at http://www.molpharm.org

ABSTRACT

Recently, the novel synthetic retinoid 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid (AHPN) has been shown to inhibit cell growth and induce apoptosis in several human carcinoma cell lines. To understand the mechanism of AHPN action, we identified, using the differential display method, several genes that are differentially regulated by AHPN. The sequence of one of these genes was highly homologous to mouse *MyD118,* a gene closely related to *GADD45*. Both of these genes have been reported to play a role in negative growth control and apoptosis. *hMyD118* was expressed in a variety of tissues, including liver, skeletal muscle, kidney, pancreas, spleen, thymus, prostate, and peripheral blood leukocytes. The levels of both *hMyD118* and *GADD45* mRNA was rapidly increased in a number of carcinoma cell lines after treatment with AHPN. This increase was specific for AHPN because retinoic acid, a retinoic acid receptor-selective retinoid, and an retinoid X receptor-selective retinoid were ineffective. These results suggest that this action of AHPN involves a novel mechanism that is independent of the nuclear retinoid receptors. AHPN increases the half-life of *hMyD118* and *GADD45* mRNA by >9-fold, indicating that it causes an increase in the stability of these mRNAs. The caspase inhibitor benzyloxycarbonyl-Val-Ala-Asp fluoro-methylketone (ZVAD.fmk) had no effect on the induction of hMyD118, indicating that this increase occurred independently of caspase activation. Our study demonstrates that the inhibition of cell growth by AHPN is accompanied by an increase in *hMyD118* and *GADD45* mRNA, and that this enhancement is regulated at a post-transcriptional level. Our results support a role for *MyD118* and *GADD45* in the negative growth control by AHPN.

Retinoids, structural analogs of vitamin A, are involved in the regulation of differentiation, morphogenesis, proliferation, and apoptosis. Many but not all of these effects appear to be mediated by the nuclear retinoic acid receptors (RARs) and retinoid X receptors (RXRs) (Giguère, 1994). Retinoid receptors regulate transcription by binding to specific DNA sequences (retinoid response elements, RAREs, or RXREs) in the regulatory region of target genes. Retinoids can also exert anti-AP1 activity, and although this action is mediated by retinoid receptors, it does not involve RAREs or RXREs (Fanjul et al., 1994). However, a number of effects induced by retinoids occur independently of RARs or RXRs. For example, the growth-stimulatory effects of retroretinols on lymphocytes and fibroblasts are not mediated by retinoid receptors but through a novel yet unidentified signaling pathway (Buck et al., 1991; O'Connell et al., 1996). Several of the effects of the synthetic retinoid 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid (AHPN, also referred to as CD437) have been found to inhibit the growth and induce apoptosis of a variety of carcinoma cell lines (Shao et al., 1995; Schadendorf et al., 1996; Chao et al., 1997; Hsu et al., 1997a,b; Lu et al., 1997; Oridate et al., 1997; Sun et al., 1997a,b; Adachi et al., 1998a,b; Li et al., 1998; Meister et al., 1998). Although this retinoid binds RARγ selectively, the molecular mechanism by which AHPN induces apoptosis in carcinoma cells does not involve this receptor but is mediated by a yet unknown mechanism.

Lung cancer represents one of the leading causes of death (Mannino et al., 1998). Although many lung cancers are

[1] Present address: Department of Obstetrics and Gynecology, Osaka University Medical School, 2-2 Yamada-oka, Suita City, Osaka 565-0871, Japan.

ABBREVIATIONS: RAR, retinoic acid receptor; RXR, retinoid X receptor; RARE, retinoic acid response element; AHPN, 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid; MAP, mitogen-activated protein; PD169316, [4-(4-Fluorophenyl)-2-(4-nitrophenyl)-5-(4-pyridyl)-1H-imidazole]; CAT, chloramphenicol acetyltransferase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ZVAD.fmk, benzyloxycarbonyl-Val-Ala-Asp fluoro-methylketone.

MOLECULAR PHARMACOLOGY
aspet
Downloaded from molpharm.aspetjournals.org by guest on December 1, 2012

MOL PHARM

MOLECULAR PHARMACOLOGY

sensitive to initial chemotherapy, in many cases they become rapidly resistant. In contrast to normal cells, many lung carcinoma cell lines have been reported to be rather resistant to the growth-inhibitory effects of retinoic acid (Geradts et al., 1993; Kalemkerian et al., 1994, 1995; Sun et al., 1997a,b; Zhang and Jetten, 1997; Adachi et al., 1998b). However, the synthetic retinoids 4-(hydroxyphenyl)retinamide and AHPN have been shown to inhibit cell proliferation and induce apoptosis in several lung cancer cell lines (Kalemkerian et al., 1995; Adachi et al., 1998b; Li et al., 1998; Zou et al., 1998). Recently, AHPN has been shown to very effectively inhibit the growth of lung tumors in mice, indicating their potential usefulness in chemotherapy (Lu et al., 1997).

In this study, we used the differential mRNA display method (Liang et al., 1994) to identify changes in gene expression that are related to AHPN-induced growth arrest and apoptosis in human lung carcinoma H460 cells. Several AHPN-inducible genes were identified and cloned by this method. The sequence of one of these genes exhibited high homology to the murine myeloid differentiation primary response gene *MyD118* (Abdollahi et al., 1991) and likely represents the human homolog, which has not been described previously. *MyD118* is closely related to the growth arrest and the DNA damage gene *GADD45* (Fornace, 1992). *mMyD118* and *GADD45* encode nuclear proteins that interact with proliferating cell nuclear antigen and the cyclin-dependent kinase inhibitor $p21^{WAF1/Cip1}$ (Smith et al., 1994; Vairapandi et al., 1996; Prosperi, 1997). Increased expression of *MyD118* and *GADD45* have been shown to suppress cell growth (Zhan et al., 1994). These studies suggest that these proteins have an important function in negative growth control and apoptosis.

We demonstrate that AHPN enhanced the level of both *hMyD118* and *GADD45* mRNA in a variety of carcinoma cell lines. It is likely that the increase in these growth-suppressing genes contribute to the growth-inhibitory effects and the induction of apoptosis by AHPN. Our results also indicate that the increase in the level of *hMyD118* and *GADD45* mRNA by AHPN is regulated at the post-transcriptional level and is largely due to an increased RNA stability. The induction of *hMyD118* and *GADD45* expression is highly specific for AHPN, in agreement with the concept that this action is not mediated by the nuclear retinoid receptors RARs and RXRs but via a novel signaling pathway.

Experimental Procedures

Materials. The retinoid AHPN (also referred to as CD437) was described previously (Delescluse et al., 1991) and obtained from Dr. U. Reichert (CIRD Galderma, Valbonne, France). The RAR- (SRI-6751–84/TTAB, 4-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-anthracenyl)-benzoic acid), RXR- (SR11217, 4-[1-(5,6,7,8-tetrahydro-5,5,8,8,-tetramethyl-2-naphthalenyl)-2-methylpropenyl]benzoic acid), and anti-AP1-selective [SR11302, (*E*)-3-methyl-9-(2,6,6-trimethylcyclohexenyl)-7-(4-methyl-phenyl)-2,4,6,8-nonatetraenoic acid] retinoids (Lehmann et al., 1992; Fanjul et al., 1994) were provided by Dr. M. Dawson (SRI, Menlo Park, CA). All-*trans*-retinoic acid was obtained from Hoffmann-La Roche (Nutley, NJ). Retinoids were dissolved in dimethyl sulfoxide (DMSO). Control cells received DMSO only. Cycloheximide and actinomycin D were purchased from Sigma Chemical Co. (St. Louis, MO). The cell-permeable, irreversible caspase inhibitor benzyloxycarbonyl-Val-Ala-Asp fluoro-methylketone (ZVAD.fmk) and the selective p38 mitogen-activated protein (MAP) kinase inhibitor PD169316 ([4-(4-Fluorophenyl)-2-(4-nitrophenyl)-5-(4-pyridyl)-11+-imidazole]) were purchased from Calbiochem (San Diego, CA).

Cell Culture. The human lung carcinoma cell lines H441, H460, A549, H1355, and Calu-6 and the human mammary carcinoma cell line MCF-7 were obtained from American Type Culture Collection (Rockville, MD). The human fibrosarcoma Ht1080 cell lines containing either wild-type or mutant p53, bladder carcinoma T24, and colorectal carcinoma cell line RKO were obtained from Dr. Gloria Preston (National Institute of Environmental Health Sciences). All cell lines except for Ht1080 and RKO were maintained in RPMI 1640 supplemented with 10% fetal bovine serum, penicillin, and streptomycin. The Ht1080 and the RKO cell lines were grown in Dulbecco's modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum. All cell lines were *Mycoplasma* free.

Differential Display. Differential mRNA display was performed essentially as described by Liang et al. (1994). H460 cells were treated with 2.5 μM AHPN or vehicle (DMSO). After 8 h of treatment, total RNA was isolated. RNA was reverse-transcribed to cDNA with random hexamers. Polymerase chain reaction (PCR) was performed with a 5′-end primer containing a *Hin*dIII site, an one-base anchored oligo-$(dT)_{11}$ primer (Liang et al., 1994) including a *Bam*HI site, [α-^{33}P]dCTP (1000–3000 Ci/mmole; Amersham Life Sciences, Arlington Heights, IL), and Expand High Fidelity polymerase (Boehringer Mannheim, Indianapolis, IN). PCR products were separated on a 6% polyacrylamide/8 M urea sequencing gel. Differentially displayed bands were recovered from the polyacrylamide gel and then reamplified with the same primer pair. Reamplified products were used as probes to confirm differential expression by Northern blot analysis and then cloned into pGEM-3Z (Promega Corp., Madison, WI).

cDNA Library Screening. To obtain full-length cDNA clones, a human heart cDNA library (Stratagene, La Jolla, CA) was screened with a radiolabeled, reamplified band as a probe. Sequence analysis was performed in both directions by the dideoxynucleotide chain-termination method using a T7 Sequenase II kit (Amersham, Cleveland, OH). Nucleotide sequences were compared with those in Genbank using BLAST (National Center for Biotechnology Information, Bethesda, MD).

Northern Blot Analysis. RNA from cultured cells was isolated using Tri-Reagent (Sigma Chemical Co., St. Louis, MO) according to the manufacturer's protocol. Poly(A)$^+$ RNA was isolated from total RNA with oligo(dT) columns (Qiagen, Chatsworth, CA). Total RNA (30 μg) was electrophoresed through a 1.2% denaturing agarose-formaldehyde gel as described (Adachi et al., 1998b), blotted to a Nytran Plus membrane (Schleicher & Schuell, Keene, NH), and UV-cross-linked. Hybridizations were performed for 1–2 h at 68°C using QuikHyb reagent (Stratagene, La Jolla, CA); blots were washed twice with 2× SSC, 0.05% SDS at room temperature for 15 min. The final wash was with 0.5× standard saline citrate, 0.1% SDS for 30 min at 65°C. Autoradiography was carried out with Hyperfilm-MP (Amersham) at −70°C using double intensifying screens. The cDNA probe for *hMyD118* that was isolated by this differential display experiment was used as a probe for Northern analysis. The cDNA for *GADD45* was obtained from Dr. A.J. Fornace (National Institutes of Health, Bethesda, MD). Tissue distribution was determined with a human multiple tissues RNA blot (Clontech, Palo Alto, CA).

Transfection. Reporter gene constructs containing different regions of the upstream *GADD45*promoter region in the vector p CAT-Basic (Promega) were obtained from Dr. A.J. Fornace. The construct pdc45-40 contains the region from −2578 to 149, pdc45-20 contains the region from −848 to 149, and pdc45-24 contains the region from −41 to 149 (Hollander et al., 1993). A549 cells (2×10^5/well) were plated into six-well dishes 16 h before transfection. Reporter plasmids and a reference β-actin-luciferase plasmid were transfected into cells by 1,3-di-oleoyloxy-2-(6-carboxy-spermyl)-propylamid (DOSPER, Boehringer Mannheim) according to the manufacturer's

Downloaded from molpharm.aspetjournals.org by guest on December 1, 2012

protocol. Twenty h after transfection, cells were treated with AHPN or DMSO, and 24 h later, cells were assayed for CAT and luciferase. CAT protein was measured using a CAT-ELISA kit (Boehringer Mannheim). Luciferase activity was assayed with a luciferase assay kit (Promega) in a Lumat LB9501 luminometer (Berthold). The relative level of CAT protein was normalized for luciferase activity.

RNA Stability Assay. H460 cells grown for 8 h in the presence or absence of 2.5 μM AHPN were treated with actinomycin D (5 μg/ml), and at different time intervals, cells were collected, and RNA was isolated. For RNA stability assay, total RNA from AHPN-treated cells and poly(A)$^+$ RNA from untreated H460 cells were examined by Northern blot analysis using radiolabeled probes for *hMyD118, GADD45,* or glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*). Hybridization signals were quantitated with a PhosphorImage analyzer (STROM 860; Molecular Dynamics, Sunnyvale, CA) using ImageQuant software (Molecular Dynamics). mRNA half-life ($T_{1/2}$) was calculated using the equation $C = C_0e^{Kdt}$, where C is the remaining mRNA level at time point t, C_0 is the amount of RNA at zero time of actinomycin D addition, and Kd is the mRNA decay constant.

Results

Differential mRNA Display Analysis. To obtain additional insight into the mechanism of action of AHPN, differential mRNA display analysis was used to identify specific response genes induced by AHPN in human lung carcinoma cells. For this purpose, exponentially growing cultures of human lung carcinoma H460 cells were treated with or without AHPN, and after 8 h of treatment, total RNA was extracted. cDNA was synthesized by reverse transcriptase reaction and amplified with one-base anchored oligo(dT)$_{11}$ and 5′-end primers. Radiolabeled PCR products were separated on a sequencing gel and analyzed by autoradiography. Differentially expressed cDNA fragments were recovered from the polyacrylamide gel and reamplified with the same pair of primers; their differential expression was confirmed by Northern blot analysis. The fragments, for which differential expression was confirmed, were subjected to sequence analysis. The obtained nucleotide sequences were compared with those in Genbank. One sequence, 0.4 kb in size, had high homology to the sequence in the 3′-untranslated region of the *mMyD118* gene (Abdollahi et al., 1991). To clone the full-length cDNA, a human heart cDNA library was screened with the radiolabeled 0.4 kb probe, and the inserts of the obtained clones were sequenced. The nucleotide sequence of the full-length cDNA is shown in Fig. 1. The nucleotide sequence (Genbank accession no. AF090950) was 86% identical with that of the *mMyD118* gene. *hMyD118* encodes a M_r 18,000 protein that exhibited a 90.6% identity with mMyD118 (Fig. 2). hMyD118 protein exhibited a 56.3% identity with the closely related gene *GADD45* (Fig. 2).

Tissue-Specific Expression of *hMyD118*. We next analyzed the expression of *hMyD118* in several normal human tissues. Northern blot analysis showed that *hMyD118* was expressed in many tissues including heart, placenta, liver skeletal muscle, kidney, pancreas, spleen, thymus, prostate, uterus, and peripheral blood leukocytes (Fig. 3). *MyD118* mRNA expression was very low in brain, lung, pancreas, and testis. Our results show that *MyD118* is much more widely expressed than originally reported (Abdollahi et al., 1991).

AHPN was able to induce *MyD118* mRNA expression in a variety of cell lines. AHPN strongly induced *MyD118* mRNA expression in human lung carcinoma H441, H460, A549, and H1355 cell lines and to a lesser extent in Calu-6 cells. AHPN also strongly increased expression of the closely related gene *GADD45* in H441, H460, A549, and H1355 cell lines, whereas *GADD45* mRNA was induced only weakly in Calu-6 cells (Fig. 4A). AHPN also increased expression of *MyD118* and *GADD45* mRNA in colorectal carcinoma RKO and bladder carcinoma T24 cells, whereas in mammary carcinoma MCF-7

```
  1 CCGCATCCACTGTGGATTATAATTGCAACATGACGCTGGAAGAGCTCGTGGCGTGCGACA
  1                              M  T  L  E  E  L  V  A  C  D  N

 60 ACGCGGCGCAGAAGATGCAGACGGTGACCGCCGCGGTGGAGGAGCTTTTGGTGGCCGCTC
 12  A  A  Q  K  M  Q  T  V  T  A  A  V  E  E  L  L  V  A  A  Q

120 AGCGCCAGGATCGCCTCACAGTGGGGGTGTACGAGTCGGCCAAGTTGATGAATGTGGACC
 22  R  Q  D  R  L  T  V  G  V  Y  E  S  A  K  L  M  N  V  D  P

180 CAGACAGCGTGGTCCTCTGCCTCTTGGCCATTGACGAGGAGGAGGAGGATGACATCGCCC
 42  D  S  V  V  L  C  L  L  A  I  D  E  E  E  E  D  D  I  A  L

240 TGCAAATCCACTTCACGCTCATCCAGTCCTTCTGCTGTGACAACGACATCAACATCGTGC
 62  Q  I  H  F  T  L  I  Q  S  F  C  C  D  N  D  I  N  I  V  R

300 GGGTGTCGGGCAATGCGCGCCTGGCGCAGCTCCTGGGAGAGCCGGCCGAGACCCAGGGCA
 82  V  S  G  N  A  R  L  A  Q  L  L  G  E  P  A  E  T  Q  G  T

360 CCACCGAGGCCCGAGACCTCCACTGTCTTCCCTTCCTACAGAACCCTCACACGGACGCCT
102  T  E  A  R  D  L  H  C  L  P  F  L  Q  N  P  H  T  D  A  W

420 GGAAGAGCCACGGCTTGGTGGAGGTGGCCAGCTACTGCGAAGAAAGCCGGGGCAACAACC
122  K  S  H  G  L  V  E  V  A  S  Y  C  E  E  S  R  G  N  N  Q

480 AGTGGGTCCCCTACATCTCTCTTCAGGAACGCTGAGGCCCTTCCCAGCAGCAGAATCTGT
142  W  V  P  Y  I  S  L  Q  E  R

540 TGAGTTGCTGCCAACAAACAAAAAATACAATAAATATTTGAACCCCCTCCCCCCCAGCAC
600 AACCCCCCCAAAACAACCCAACCCACGAGGACCATCGGGGGCAGGTCGTTGGAGACTGAA
660 GAGAAAGAGAGAGAGGAGAAGGGAGTGAGGGGCCGCTGCCGCCTTCCCCATCACGGAGGG
720 TCCAGACTGTCCACTCGGGGGTGGAGTGAGACTGACTGCAAGCCCCACCCTCCTTGAGAC
780 TGGAGCTGAGCGTCTGCATACGAGAGACTTGGTTGAAACTTGGTTGGTCCTTGTCTGCAC
840 CCTCGACAAGACCACACTTTGGGACTTGGGAGCTGGGGCTGAAGTTGCTCTGTACCCATG
900 AACTCCCAGTTTGCGAATTAATAAGAGACAATCTATTTTTGTTACTTGCACTTGTTATTCG
960 AACCACTGAGAGCGAGATGGGAAGCATAGATATCTATATTTTTATTTCTACTATGAGGGC
1020 CTTGTAATAAATTTCTAAAGCCTC(A)n
```

Fig. 1. The nucleotide and deduced amino acid sequence of *hMyD118* (Genbank accession no. AF090950). The amino acid sequence is noted below the nucleotide sequence, and the translation start and stop codons are shown in bold. The polyadenylation signal is underlined.

```
hMyD118 : MTLEELVACDNAAQKMQTVTAAVEELLVAAQRQDRLTVGVYESAKLMNVDPDSVVLCLLA
mMyD118 : ........S...V....A......Q.................A.................
hGADD45 : .....FS.GEQKTER.DK.GD.L..V.SK.LS.RTI......A...L.....N.......

hMyD118 : IDEEEEDDIALQIHFTLIQSFCCDNDINIVRVSGNARLAQLLGE-----PAETQGTTEAR
mMyD118 : ........................D......MQ.........-------....L......
hGADD45 : A..DDDR.V..........A...E.....L...NPG...E..LLETDAG..ASE.AEQPP

hMyD118 : DLHCLLVTNPHTDAWKSHGLVEVASYCEESRGNNQWVPYISLQER
mMyD118 : .........C...S...Q.............................
hGADD45 : ....V.......SSQ..DPA.SQLICF.R...YMD...........
```

Fig. 2. Comparison of the amino acid sequences of hMyD118, mMyD118, and GADD45. Amino acids that are identical with those in hMyD118 sequence are shown as dots, whereas sequence differences are indicated.

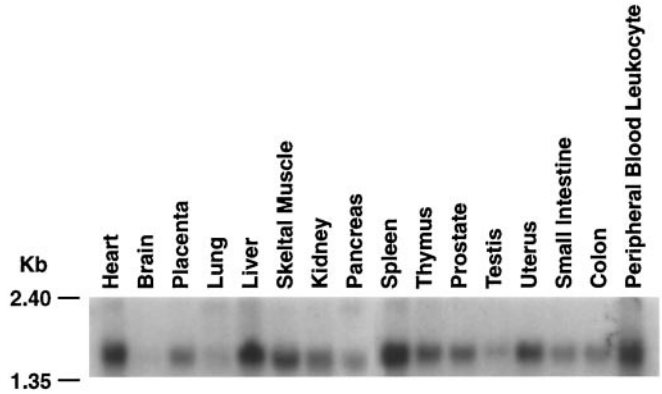

Fig. 3. Tissue-specific expression of *hMyD118*. Northern blot analysis was performed on total RNA isolated from different human tissues using a radiolabeled probe for *hMyD118*. The source of the RNA is indicated above each lane. The size of *MyD118* mRNA is shown on the left.

MOL PHARM
MOLECULAR PHARMACOLOGY
aspet
Downloaded from molpharm.aspetjournals.org by guest on December 1, 2012

cells, it increased expression of *GADD45* but not *MyD118* mRNA (Fig. 4B). Because *GADD45* has been reported to be regulated by p53 (Smith et al., 1994), we compared the induction of *GADD45* by AHPN in two human fibrosarcoma Ht1080 cell lines containing either wild-type p53 or mutant p53. As shown in Fig. 4B, Ht1080(p53wt) cells expressed high basal levels of *MyD118* and *GADD45* mRNA, which were only slightly increased after AHPN treatment. In contrast, Ht1080(p53 mut) expressed low basal levels of *MyD118* and *GADD45* mRNA, which increased 3- and 7-fold, respectively, after treatment with AHPN. These findings suggest that the induction of *MyD118* and *GADD45* by AHPN is not dependent on p53. This conclusion is supported by the observed increase in *MyD118* and *GADD45* mRNA by AHPN in H1355 and 441 cells, which also contain p53 mut (Adachi et al., 1998b). However, the difference in the basal level of *MyD118* and *GADD45* mRNA expression between Ht1080(p53wt) and Ht1080(p53 mut) may involve a p53-dependent control mechanism.

Specificity of AHPN Action. To determine the specificity of the action of AHPN, we compared the effect of various retinoids on *hMyD118* expression in H460 and A549 cells. Cells were treated with the indicated retinoid; after 8 h, cells were collected, and RNA was isolated and examined by Northern analysis. Figure 5 shows that treatment of H460 and A549 with AHPN greatly increases the level of *MyD118* and *GADD45* mRNA expression. Two retinoids, TTAB and SR11217, which bind and activate selectively RAR or RXR receptors, respectively, did not increase *MyD118* or *GADD45* expression, suggesting that activation of RARs but not that of RXRs is sufficient for this action. Many retinoids have been reported to exhibit anti-AP1 activity, including TTAB and SR11217. The fact that SR11217 did not increase the level of *MyD118* and *GADD45* mRNA indicates that this action does not require the anti-AP-1 activity of retinoids. The latter is supported by the observation showing that the retinoid SR11302 with reported selective anti-AP-1 activity (Fanjul et al., 1994) also did not have any effect on the expression of these genes. These results demonstrate that the induction of *hMyD118* and *GADD45* mRNA is highly specific for AHPN, as has been reported for the growth-inhibitory activity of these retinoids (Adachi et al., 1998b). These findings support the concept that these actions of AHPN do not involve retinoid receptors but are mediated by a novel mechanism.

We next examined the time course of the induction of *MyD118* and *GADD45* by AHPN. In H460 cells, the induction of *MyD118* mRNA by AHPN was transient. *MyD118* mRNA was induced within 4 h but then decreased after >8 h of AHPN treatment (Fig. 6, A and C). In contrast, in A549 cells AHPN caused a steady increase in *MyD118* mRNA levels over a period of 24 h treatment (Fig. 6, B and D). In both

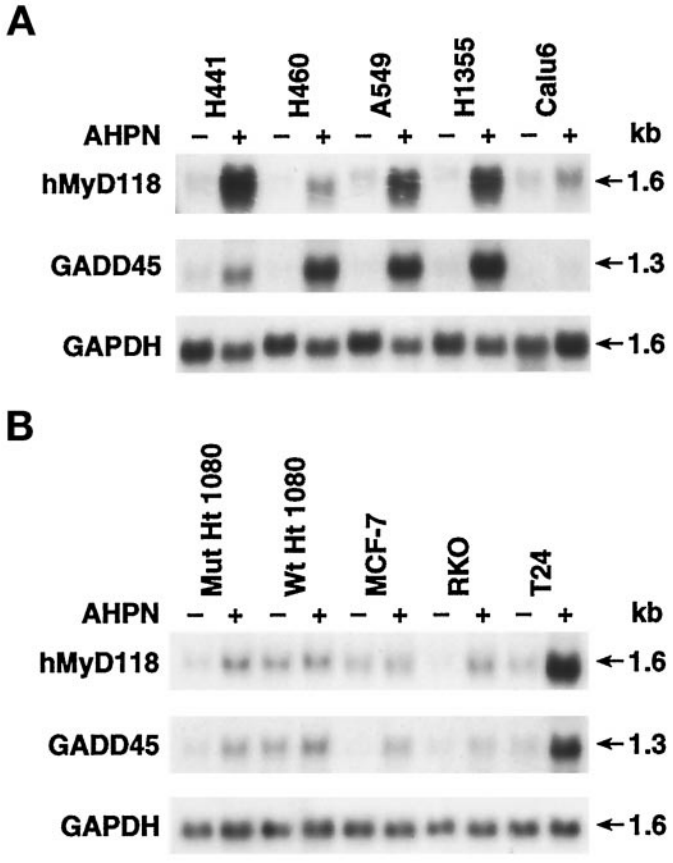

Fig. 4. Comparison of the induction of *hMyD118* and *GADD45* by AHPN in several cell lines. A, human lung carcinoma H441, H460, A549, H1355, and Calu-6 cells were treated for 24 h with 1 μM AHPN. Total RNA was isolated and examined by Northern blot analysis using radiolabeled probes for *hMyD118, GADD45,* and *GAPDH*. B, human fibrosarcoma MutHt 1080 and WtHt 1080 cells, mammary carcinoma MCF-7, colorectal carcinoma RKO, and bladder carcinoma T24. Cells were treated for 16 h with AHPN.

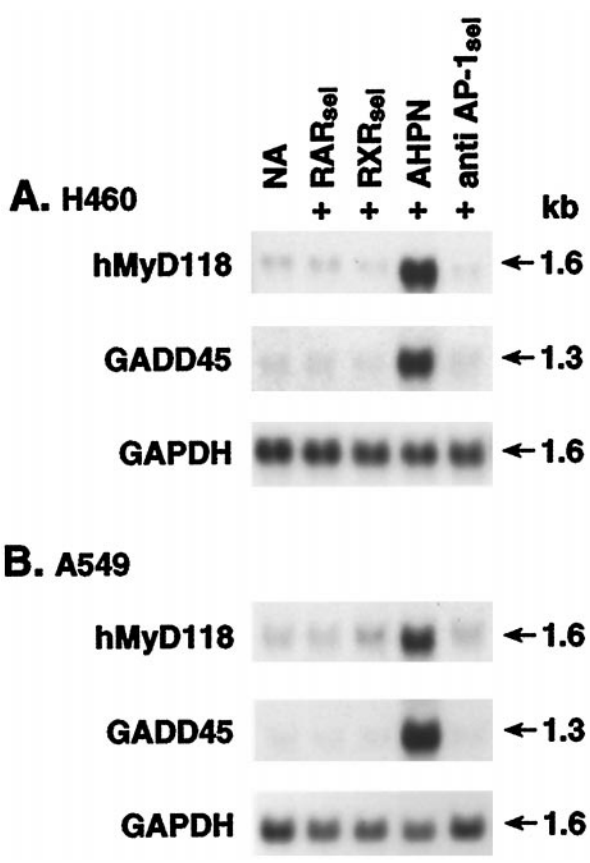

Fig. 5. Effect of various retinoids on the expression of *hMyD118* and *GADD45* mRNA in lung carcinoma H460 and A549 cells. Cells were treated for 8 h with 1 μM for the indicated retinoid. Total RNA was isolated and examined by Northern blot analysis using radiolabeled probes for *hMyD118, GADD45,* and *GAPDH*. A, RNA from H460 cells treated with DMSO (0.1%; NA), RAR-selective retinoid (RAR_{sel}), RXR-selective retinoid (RXR_{sel}), AHPN, and anti AP-1 selective retinoid (anti $AP\text{-}1_{sel}$). B, RNA from A549 cells treated with the same retinoids.

MOL PHARM
MOLECULAR PHARMACOLOGY
aspet
Downloaded from molpharm.aspetjournals.org by guest on December 1, 2012

MOL PHARM
MOLECULAR PHARMACOLOGY
aspet

H460 and A549 cells, *GADD45* mRNA increased after 4 h of AHPN treatment. The induction of *MyD118* mRNA in H460 cells was somewhat faster than that of *GADD45* mRNA (Fig. 6C).

Induction of *MyD118* and *GADD45* mRNA Is Independent of Activation of Caspases or p38 MAP Kinase. Previous studies have shown that the induction of apoptosis by AHPN is associated with an increase in activity of specific cysteine proteases, named caspases (Piedrafita and Pfahl, 1997; Adachi et al., 1998a). To determine whether activation of caspases is required for the induction of *MyD118* by AHPN, we examined the effect of the caspase inhibitor ZVAD.fmk on *MyD118* expression. Concentrations of ZVAD.fmk that totally inhibited caspase activity (Adachi et al., 1998a) had no effect on the AHPN-dependent increase of *MyD118* mRNA (Fig. 7A). These results indicate that the increase in *MyD118* does not require the activation of caspases. Piedrafita and Pfahl (1997) have shown that the induction of caspase is independent of protein synthesis. It is therefore likely that the increase in *MyD118* and caspase activation occur independently from each other.

Treatment of H460 cells with AHPN induces a rapid (within 1 h) phosphorylation/activation of the MAP kinase p38 (Sakaue et al., manuscript in preparation). To determine whether this activation was required for the induction of *MyD118* or *GADD45* mRNA expression, H460 cells were treated with AHPN in the presence or absence of the inhibitor PD169316. As shown in Fig. 7B, the MAP kinase inhibitor did not effect the induction of *MyD118* or *GADD45* mRNA by AHPN. These results indicate that p38 activation is not required for *MyD118*/*GADD45* induction and suggest that these events are uncoupled.

Regulation of *MyD118* and *GADD45* by AHPN. To determine at what level AHPN regulates the expression of

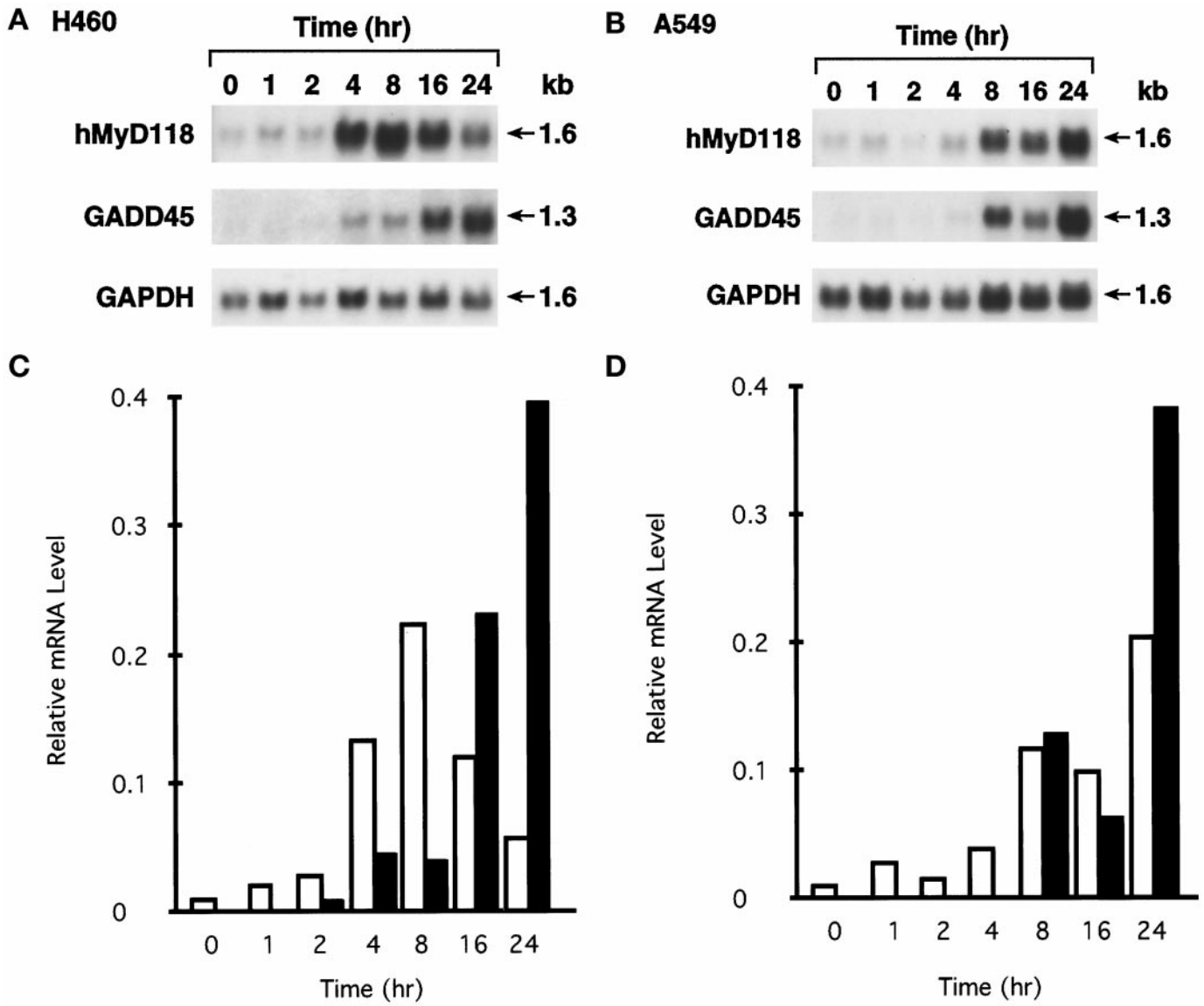

Fig. 6. Time course of the induction of *hMyD118* and *GADD45* mRNA by AHPN in lung carcinoma H460 and A549 cells. Cells were treated with 1 μM AHPN for the time period indicated. Total RNA was isolated and examined by Northern blot analysis using radiolabeled probes for *hMyD118*, *GADD45*, and *GAPDH*. Hybridization signals were quantitated with a PhosphorImage analyzer using ImageQuant software as described in *Experimental Procedures*. The results are plotted as the relative level of *hMyD118* (open bars) or *GADD45* mRNA (solid bars). H460 (A and C) and A549 (B and D) cells treated with AHPN.

Downloaded from molpharm.aspetjournals.org by guest on December 1, 2012

these genes, we examined the effect of AHPN on the transcriptional activation from a *GADD45* promoter regulatory region. Transcription of the reporter form a −2578/149 or a −848/149 *GADD45* promoter region was very efficient (not shown). However, AHPN had little effect on this transcriptional activation, suggesting that it does not control the transcription of this gene through elements contained in this region.

To determine whether *MyD118* and *GADD45* were regulated by AHPN at the post-transcriptional level, we examined the effect of AHPN on RNA stability. H460 cells were treated for 8 h with AHPN or vehicle (DMSO); transcription of RNA was then inhibited by the addition of actinomycin D. At different time intervals, total RNA (from AHPN-treated cells) or poly(A)$^+$ RNA (from control cells) was isolated and examined by Northern blot analysis using probes for *MyD118, GADD45,* and *GAPDH*. As shown in Fig. 8A, the level of *MyD118* and *GADD45* mRNA in control H460 cells rapidly decreased within 2 h, whereas RNAs from AHPN-treated cells were rather stable over this time period. The level of *GADPH* mRNA in both control and AHPN-treated H460 cells remained stable over the time period tested. The relative level of *MyD118* and *GADD45* mRNA was determined and plotted, and the RNA-half-life time was calculated (Fig. 8, B and C). The half-life of *hMyD118* in untreated cells was 46 min compared with 7 h in AHPN-treated cells. The half-life for *GADD45* in control and AHPN-treated cells was calculated as 50 min and >8 h, respectively. These results demonstrate that AHPN controls *MyD118* and *GADD45* mRNA expression by dramatically increasing RNA stability.

As shown for myeloid leukemia cells (Selvakumaran et al., 1994), treatment of lung carcinoma H460 cells with cycloheximide also caused a dramatic increase in the level of *hMyD118* and *GADD45* mRNAs, suggesting a mechanism of regulation at a post-transcriptional level; however, no superinduction was observed when cells were treated simultaneously with cycloheximide and AHPN (not shown).

Discussion

The synthetic retinoid AHPN has been demonstrated to induce growth arrest and apoptosis in many carcinoma cells, including human lung, cervical, and mammary carcinoma and lymphoma cell lines (Shao et al., 1995; Chao et al., 1997; Lu et al., 1997; Oridate et al., 1997; Sun et al., 1997a, b; Li et al., 1998; Adachi et al., 1998a, b). Although AHPN has been demonstrated to bind RARγ selectively and to weakly induce transcriptional activation via this receptor, several studies have demonstrated that RAR or RXR nuclear retinoid receptor pathways are not involved in AHPN-induced growth arrest and apoptosis (Shao et al., 1995; Hsu et al., 1997b; Piedrafita and Pfahl, 1997; Sun et al., 1997a; Adachi et al., 1998a, b). In an effort to understand the mechanism by which AHPN induces growth arrest and apoptosis, we began to identify genes that are induced after treatment with AHPN using differential display. One of the differentially expressed clones isolated encodes the human *MyD118,* based on its high sequence homology with murine *MyD118* (Abdollahi et al., 1991). The mouse homolog was cloned previously as a novel primary response gene from terminally differentiating murine myeloid M1 cells after stimulation with multiple cytokines, including interleukin 1 and leukemia inhibitory factor. Both murine and human *MyD118* encode a 1.3-kb mRNA and a M_r 18,000 nuclear protein. In contrast to previous reports on *mMyD118* (Abdollahi et al., 1991), *hMyD118* was expressed in all tissues tested, including liver, spleen, kidney, and heart. In addition, we show that *hMyD118* mRNA is expressed at low levels in many carcinoma cell lines and is dramatically induced by AHPN. These results suggest a much wider role for *MyD118* than originally believed.

MyD118 is closely related to the growth arrest- and DNA damage-inducible gene *GADD45*. *GADD45* is induced in mammalian cells by a wide range of stimuli, including environmental stress, genotoxic drugs, and ionizing irradiation (Fornace et al., 1989, 1992). Although these genes are regulated by different mechanisms, they are often coordinately expressed and can function cooperatively in inhibiting cell growth (Zhan et al., 1994). In this study, we demonstrate that growth inhibition and induction of apoptosis by AHPN is associated with increased levels of *MyD118* and *GADD45* mRNA. Induction of *GADD45* was recently also demonstrated in AHPN-treated myoblastic leukemia HL-60 cells (Hsu et al., 1997b). In most carcinoma cell lines tested, the increase in *MyD118* and *GADD45* mRNA by AHPN occurs in parallel. However, in mammary carcinoma MCF-7 cells, AHPN enhanced *GADD45* but not *MyD118* mRNA, whereas in lung carcinoma Calu-6 cells, *GADD45* mRNA levels in-

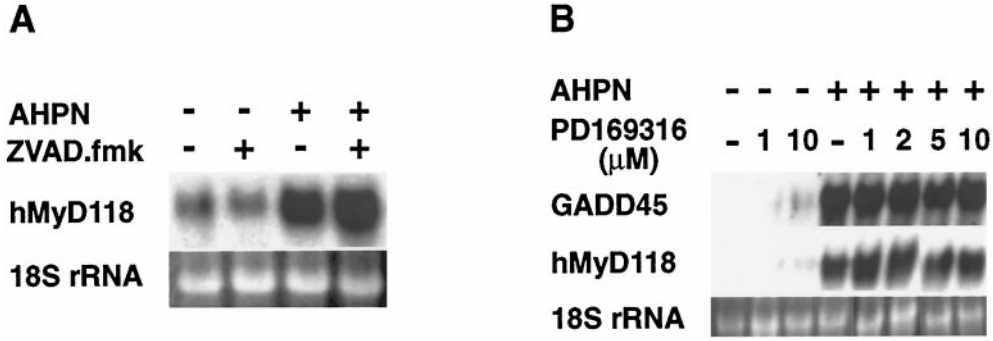

Fig. 7. The induction of *MyD118* and *GADD45* mRNA by AHPN is independent of the activation of caspases or p38 MAP kinase. A, H460 cells were treated with AHPN in the presence or absence of the caspase inhibitor ZVAD.fmk (30 μM). After 8 h of treatment, RNA was isolated and examined by Northern analysis for *hMyD118* mRNA expression. The level of 18S RNA shows equal loading of RNA. B, H460 cells were treated with AHPN in the presence or absence of the MAP kinase inhibitor PD169316 at the concentrations indicated. After 8 h of treatment, RNA was isolated and examined by Northern analysis for *hMyD118* and *GADD45* mRNA expression. The inhibition of p38 MAP kinase phosphorylation at 1 μM PD169316 was 82%, and at all other concentrations, >99%.

MOL PHARM
MOLECULAR PHARMACOLOGY
aspet
Downloaded from molpharm.aspetjournals.org by guest on December 1, 2012

creased only weakly. In H460 cells, the increase in *MyD118* mRNA is transient, whereas that of *GADD45* is not, suggesting different mechanisms of regulation. The enhancement in both *hMyD118* and *GADD45* is highly specific for AHPN because an RAR-panagonist and RXR-panagonist were unable to induce *GADD45* and *hMyD118* mRNA. This action of AHPN also does not involve the anti-AP-1 activity of retinoids because an anti-AP-1-selective retinoid and the RXR-selective retinoid, which also exhibits anti-AP-1 activity, did not increase *GADD45* or *hMyD118* mRNA. These results support previous conclusions that changes associated with AHPN growth arrest and apoptosis are not mediated by nuclear retinoid RAR and RXR receptors but through a novel signaling pathway.

Several reports have provided evidence indicating that *GADD45* and *MyD118* play a critical role in the control of cell cycle progression and apoptosis (Abdollahi et al., 1991; Fornace, 1992; Zhan et al., 1994, Smith et al., 1994; Selvakumaran et al., 1994; Vairapandi et al., 1996). This is illustrated by studies showing that terminal differentiation induced by various cytokines in murine myeloblastic M1 cells and the induction of growth arrest and apoptosis by transformin growth factor β is associated with a marked increase in *MyD118* expression (Abdollahi et al., 1991; Selvakumaran et al., 1994). Moreover, overexpression of these proteins has been shown to induce growth arrest and apoptosis (Zhan et al., 1994). Further support for a negative role of MyD118 and GADD45 in growth control comes from studies showing that these proteins can interact with cyclin-dependent kinase inhibitor $p21^{Cip1/Waf1}$ and proliferating cell nuclear antigen (Smith et al., 1994; Vairapandi et al., 1996; Prosperi, 1997). The association of increased MyD118 and GADD45 expression with AHPN-induced growth arrest and apoptosis in some of the cell lines (Adachi et al., 1998b) provides additional support for a negative growth-regulatory role of these proteins.

Previous studies showed that treatment of several lung and mammary carcinoma cells with AHPN causes growth arrest in the G_1 phase of the cell cycle and is associated with a decrease in Rb hyperphosphorylation and cyclin-dependent kinase activity and increases in the level of p53 and p21 (Shao et al., 1995; Li et al., 1996; Adachi et al., 1998b). However, the induction of growth arrest and apoptosis by AHPN occurs independently of the increase in p53 and p21 because AHPN is able to induce these genes very effectively in lung carcinoma H441, H1355, and Calu-6 cells, which either do not express p53 or p21 or express mutant p53 (Adachi et al., 1998b). Previous studies have shown that

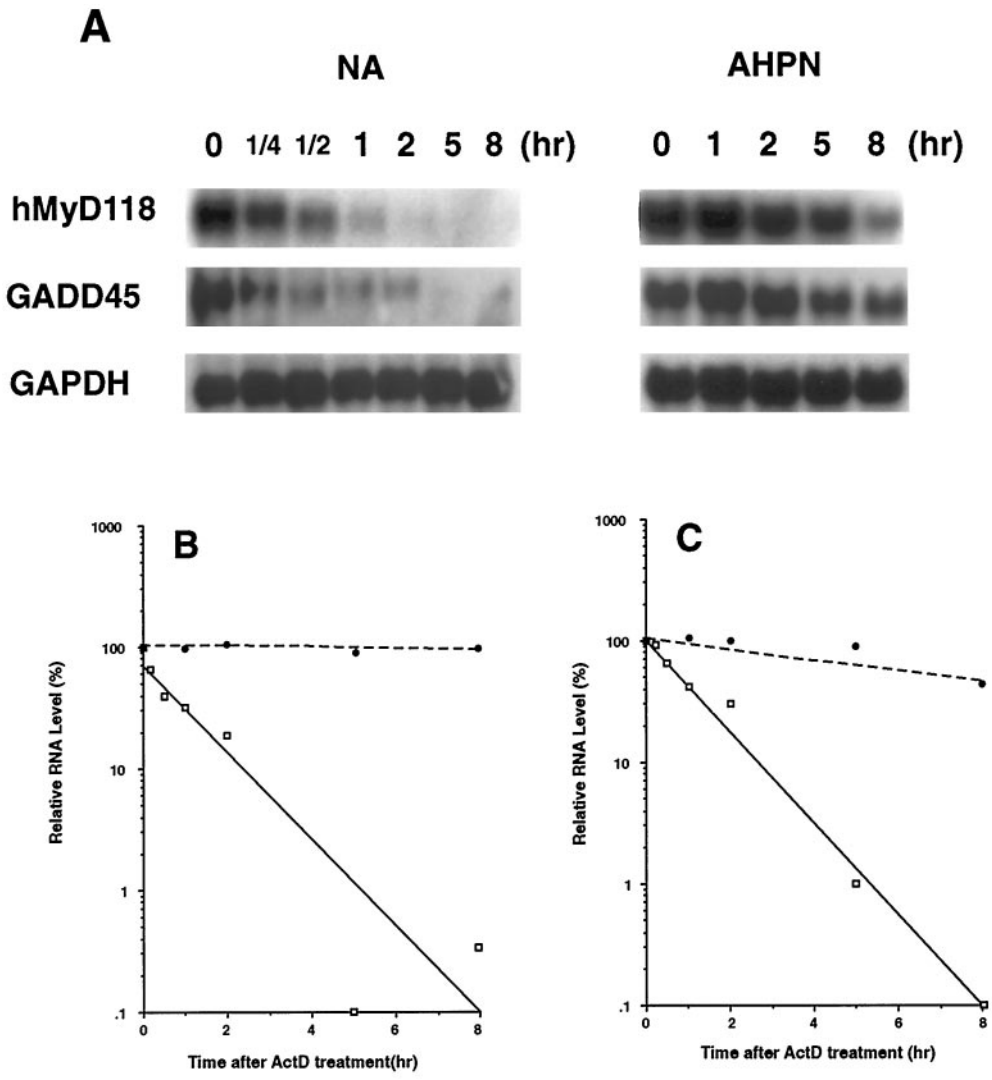

Fig. 8. Induction of *hMyD118* and *GADD45* mRNA expression by AHPN is related to increased RNA stability. A, after 8 h of treatment with AHPN or vehicle, H460 cells were treated subsequently with actinomycin D (5 μg/ml). At the indicated times, RNA was isolated and examined by Northern blot analysis with radiolabeled probes for *hMyD118, GADD45,* and *GAPDH*. Poly(A)$^+$ RNA was used in the case of control H460 cells, whereas total RNA was used in the case of AHPN-treated cells. Hybridization signals were quantitated with a PhosphorImage analyzer using ImageQuant software as described in *Experimental Procedures*. The results are plotted as the relative level of *hMyD118* (B) or *GADD45* mRNA (C) as the percentage of the amount at time 0 of actinomycin D addition.

MOL PHARM
MOLECULAR PHARMACOLOGY
aspet
Downloaded from molpharm.aspetjournals.org by guest on December 1, 2012

GADD45 can be regulated in a p53-dependent and -independent manner, but that *MyD118* is not controlled by p53 (Zhan et al., 1994). As was reported previously for p21 (Shao et al., 1995; Adachi et al., 1998b), the increase in *MyD118* and *GADD45* mRNA by AHPN is independent of p53 expression.

Little is known about the p53-independent mechanisms of *MyD118* and *GADD45* regulation. We demonstrate that the induction of *MyD118* and *GADD45* mRNA is unrelated to activation of caspases or p38 MAP kinase, two cellular responses that are rapidly induced by AHPN (Adachi et al., 1998a; Sakaue et al., manuscript in preparation). We also found no difference in the promoter activity of the upstream regulatory region of the *GADD45* gene in H460 cells treated with and without AHPN. However, AHPN caused a large increase in the half-life of both *MyD118* and *GADD45* mRNAs; their half-life was extended almost 9-fold. These results suggest that the increase in *MyD118* and *GADD45* mRNA levels by AHPN is largely regulated by a post-transcriptional mechanism and is due to increased stability of their mRNAs. RNA stability can be controlled by different mechanisms (Chen and Shyu, 1995; Ross, 1996). The family of early response genes, to which *GADD45* and *mMyD118* belong, encode highly unstable RNAs that often contain one or more adenylate/uridylate-rich instability elements in their 3′ UTR. Alternatively, mRNA-binding proteins, which bind to RNA stem-loops, may protect RNA from degradation by endonucleases and stabilize mRNAs (Ross, 1996). Future studies have to determine whether binding of specific RNA-binding protein to adenylate/uridylate-rich elements or to RNA stem-loop motifs are involved in the increased RNA stability induced by AHPN. Recently, Koonin (1997) has found thorough computer protein alignments that GADD45 and MyD118 are homologues of the eukaryotic ribosomal (r) protein S12 and belong to an ancient superfamily of ribosome-associated proteins. It has been suggested that these proteins may also possess RNA binding properties and could themselves have a role in regulating transcription and translation.

In summary, we demonstrate that the induction of growth arrest by AHPN, and in certain cell lines apoptosis, is associated with an increase in the level of *MyD118* and *GADD45* mRNA. This association is in agreement with a negative growth regulatory function of these genes. The up-regulation of these mRNAs occurs at a post-transcriptional level and is due to increased stability of the respective mRNAs. This increase appears not to be mediated by RAR or RXR receptors but by a novel signaling pathway.

References

Abdollahi A, Lord, KA, Hoffman-Liebermann B and Liebermann DA (1991) Sequence and expression of a cDNA encoding MyD118: a novel myeloid differentiation primary response gene induced by multiple cytokines. *Oncogene* **6:**165–167.

Adachi H, Adams A, Hughes FM, Zhang J, Cidlowski JA and Jetten AM (1998a) Induction of apoptosis by the novel retinoid AHPN in human T cell lymphoma cells involves caspase-dependent and independent pathways. *Cell Death Differ* **5:**973–983.

Adachi H, Preston G, Harvat B, Dawson MI and Jetten AM (1998b) Inhibition of cell proliferation and induction of apoptosis by the retinoid AHPN in human lung carcinoma cells. *Am J Respir Cell Mol Biol* **18:**323–333.

Buck J, Derguini F, Levi E, Nakanishi K and Hammerling U (1991) Intracellular signaling by 14-hydroxy-4,14-retro-retinol. *Science (Wash DC)* **254:**1654–1656.

Chao WR, Hobbs PD, Jong L, Zhang XK, Zheng Y, Wu Q, Shroot B and Dawson MI (1997) Effects of receptor class- and subtype-selective retinoids and an apoptosis-inducing retinoid on the adherent growth of the NIH:OVCAR-3 ovarian cancer cell line in culture. *Cancer Lett* **115:**1–7.

Chen CYA and Shyu AB (1995) AU-rich elements: Characterization and importance in mRNA degradation. *Trends Biochem Sci* **20:**465–470.

Delescluse C, Cavey MT, Martin B, Bernard BA, Reichert U, Maignan J, Darmon M and Shroot B (1991) Selective high affinity RA receptor α or β-γ ligands. *Mol Pharmacol* **40:**556–562.

Fanjul A, Dawson MI, Hobbs PD, Jong L, Cameron JF, Harlev E, Graupner G, Lu XP and Pfahl M (1994) A new class of retinoids with selective inhibition of AP-1 inhibits proliferation. *Nature (London)* **372:**107–111.

Fornace AJ (1992) DNA-damage inducible genes in mammalian cells. *Annu Rev Genet* **26:**505–524.

Fornace AJ, Nebert DW, Hollander MC, Luethy JD, Papathanasiou M, Fargnoli J and Holbrook NJ (1989) Mammalian genes coordinately regulated by growth arrest signals and DNA-damaging agents. *Mol Cell Biol* **9:**4196–4203.

Geradts J, Chen JY,. Russell EK, Yankaskas JR, Nieves L and Minna JD (1993) Human lung cancer cell lines exhibit resistance to retinoic acid treatment. *Cell Growth Differ* **4:**799–809.

Giguère V (1994) RA receptors and cellular retinoid binding proteins: Complex interplay in retinoid signaling. *Endocr Rev* **15:**61–79.

Hollander MC, Alamo I, Jackman J, Wang MG, McBride OW and Fornace AJJ (1993) Analysis of the mammalian *gadd45* gene and its response to DNA damage. *J Biol Chem* **268:**24385–24393.

Hsu CK, Rishi AK, Li XS, Dawson MI, Reichert U, Shroot B and Fontana JA (1997a) Bcl-X(L) expression and its downregulation by a novel retinoid in breast carcinoma cells. *Exp Cell Res* **232:**17–24.

Hsu CA, Rishi AK, Li XS, Gerald TM, Dawson MI, Schiffer C, Reichert U, Shroot B, Poirer GC and Fontana JA (1997b) Retinoid induced apoptosis in leukemia cells through a retinoic acid nuclear receptor-independent pathway. *Blood* **89:**4470–4479.

Kalemkerian GP, Jasti RK, Celano P, Nelkin BD and Mabry M (1994) All-*trans*-retinoic acid alters *myc* gene expression and inhibits in vitro progression in small lung cancer. *Cell Growth Differ* **5:**55–60.

Kalemkerian GP, Slusher R, Ramalingam S, Gadgeel S and Mabry M (1995) Growth inhibition and induction of apoptosis by fenretinide in small-cell lung cancer cell lines. *J Natl Cancer Inst* **87:**1674–1680.

Koonin EV (1997) Cell cycle and apoptosis: Possible roles of Gadd45 and MyD118 proteins inferred from their homology to ribosomal proteins. *J Mol Med* **75:**236–238.

Lehmann JM, Jong L, Fanjul A, Cameron JF, Lu XP, Haefner P, Dawson MI and Pfahl M (1992) Retinoids selective for retinoid X receptor response pathways. *Science (Wash DC)* **258:**1944–1946.

Liang P, Zhu W, Zhang X, Gao Z, O'Connell RP, Averboukh L, Wang F and Pardee AB (1994) Differential display using one base anchored oligo-dT primers. *Nucleic Acids Res* **22:**5763–5764.

Li Y, Lin B, Agadir A, Liu R, Dawson MI, Reed JC, Fontana JA, Bost F, Hobbs PD, Zheng Y, Chen GQ, Shroot B, Mercola D and Zhang XK (1998) Molecular determinants of AHPN (CD437)-induced growth arrest and apoptosis in human lung cancer cell lines. *Mol Cell Biol* **18:**4719–4731.

Li XS, Rishi AK, Shao ZM, Dawson MI, Jong L, Shroot B, Reichert U, Ordonez J and Fontana JA (1996) Posttranscriptional regulation of p21WAF1/CIP1 expression in human breast carcinoma cells. *Cancer Res* **56:**5055–5062.

Lu XP, Fanjul A, Picard N, Pfahl M, Rungta D, Nared-Hood K, Carter B, Piedrafita J, Tang S, Fabbrizio E and Pfahl M (1997) Novel retinoid-related molecules as apoptosis inducers and effective inhibitors of human lung cancer cells in vivo. *Nat Med* **3:**686–690.

Mannino DM, Ford E, Giovino GA and Thun M (1998) Lung cancer in the United States from 1979 to 1992: An analysis using multiple-cause mortality data. *Int J Epidemiol* **27:**159–166.

Meister B, Fink FM, Hittmair A, Marth C and Widschwendter M (1998) Antiproliferative activity and apoptosis induced by retinoic acid receptor-gamma selectively binding retinoids in neuroblastoma. *Anticancer Res* **18:**1777–1786.

O'Connell MJ, Chua R, Hoyos B, Buck J, Chen Y, Derguini F and Hammerling U (1996) Retro-retinoids in regulated cell growth and death. *J Exp Med* **184:**549–555.

Oridate N, Higuchi M, Suzuki S, Shroot B, Hong WK and Lotan R (1997) Rapid induction of apoptosis in human C33A cervical carcinoma cells by the synthetic retinoid 6-[3-(1-adamantyl)hydroxyphenyl]-2-naphtalene carboxylic acid (CD437). *Int J Cancer* **70:**484–487.

Piedrafita FJ and Pfahl M (1997) Retinoid-induced apoptosis and Sp-1 cleavage occur independently of transcription and require caspase activation. *Mol Cell Biol* **17:**6348–6358.

Prosperi E (1997) Multiple roles of the proliferating cell nuclear antigen: DNA replication, repair and cell cycle control. *Prog Cell Cycle Res* **3:**193–210.

Ross J (1996) Control of messenger RNA stability in higher eukaryotes. *Trends Genet* **12:**171–175.

Schadendorf D, Kern MA, Artuc M, Pahl HL, Rosenbach T, Fichtner I, Nurnberg W, Stuting S, Stebut E, Worm M, Makki A, Jurgovsky K, Kolde G and Henz BM (1996) Treatment of melanoma cells with the synthetic retinoid CD437 induces apoptosis via activation of AP-1 in vitro and causes growth inhibition in xenografts in vivo. *J Cell Biol* **135:**1889–1898.

Selvakumaran M, Lin HK, Sjin RT, Reed JC, Liebermann DA and Hoffman B (1994) The novel primary response gene *MyD118* and the proto-oncogenes *myb, myc,* and *bcl-2* modulate transforming growth factor β1-induced apoptosis of myeloid leukemia cells. *Mol Cell Biol* **14:**2352–2360.

Shao ZM, Dawson MI, Li XS, Rishi AK, Sheikh MS, Han QX, Ordonez JV, Shroot B and Fontana JA (1995) p53 independent G0/G1 arrest and apoptosis induced by a novel retinoid in human breast cancer cells. *Oncogene* **11:**493–504.

Smith ML, Chen IT, Zhan Q, Bae I, Chen CY, Gilmer TM, Kastan MB, O'Connor PM and Fornace AJJ (1994) Interaction of the p53-regulated protein gadd45 with proliferating cell nuclear antigen. *Science (Wash DC)* **266:**1376–1380.

Sun SY, Shroot B, Hong WK and Lotan R (1997a) Induction of apoptosis in human non-small cell lung carcinoma cells by the novel synthetic retinoid CD437. *J Cell Physiol* **173:**279–284.

Sun SY, Yue P, Dawson MI, Shroot B, Michel S, Lamph WW, Heyman RA, Teng M,

MOL PHARM
MOLECULAR PHARMACOLOGY
aspet
Downloaded from molpharm.aspetjournals.org by guest on December 1, 2012

Chandraratna RA, Shudo K, Hong WK and Lotan R (1997b) Differential effects of synthetic nuclear retinoid receptor-selective retinoids on the growth of human non-small cell lung carcinoma cells. *Cancer Res* **57:**4931–4939.

Vairapandi M, Balliet AG, Fornace AJ, Hoffman B and Liebermann DA (1996) The differentiation primary response gene *MyD118,* related to GADD45, encodes for a nuclear protein which interacts with PCNA and $p21^{WAF1/CIP1}$. *Oncogene* **12:**2579–2594.

Zhang LX and Jetten AM (1997) Retinoids and apoptosis, in *Advances in Organ Biology*, Vol. 3, pp. 161–196, JAI Press, London.

Zhan Q, Lord KA, Alamo I, Hollander MC, Carrier F, Ron D, Kohn KW, Hoffman B, Liebermann DA and Fornace AJ (1994) The *gadd* and *MyD* genes define a novel set of mammalian genes encoding acidic proteins that synergistically suppress cell growth. *Mol Cell Biol* **14:**2361–2371.

Zou CP, Kurie JM, Lotan D, Zou CC, Hong WK and Lotan R (1998) Higher potency of *N*-(4-hydroxyphenyl)retinamide than all-*trans*-retinoic acid in induction of apoptosis in non-small cell lung cancer cell lines. *Clin Cancer Res* **4:**1345–1355.

Send reprint requests to: Dr. Anton M. Jetten, Cell Biology Section, Laboratory of Pulmonary Pathobiology, National Institute of Environmental Health Sciences, National Institutes of Health, 111 T.W. Alexander Dr., Research Triangle Park, NC 27709. E-mail: jetten@niehs.nih.gov

MOL PHARM
MOLECULAR PHARMACOLOGY
aspet
Downloaded from molpharm.aspetjournals.org by guest on December 1, 2012