Extrahepatic Cancer Suppresses Nuclear Receptor–Regulated Drug Metabolism

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Abstract

**Purpose:** To determine the mechanisms by which tumors situated in extrahepatic sites can cause profound changes in hepatic drug clearance, contributing to altered drug response and chemotherapy resistance.

**Experimental Design:** We studied in wild-type or transgenic CYP3A4 reporter mice implanted with the murine Engelbreth–Holm–Swarm sarcoma changes in nuclear receptor and hepatic transcription factor expression and/or function, particularly related to CYP3A gene regulation.

**Results:** Repression of hepatic CYP3A induction was dramatic and associated with reduced levels of C/EBPβ isoforms, impaired pregnane X receptor, and constitutive androstane receptor function. Unexpectedly, extrahepatic tumors strongly reduced nuclear accumulation of retinoid X receptor alpha (RXRα) in hepatocytes, providing a potential explanation for impaired function of nuclear receptors that rely on RXRα dimerization. Profiling revealed 38 nuclear receptors were expressed in liver with 14 showing between 1.5- and four-fold reduction in expression in livers of tumor-bearing animals, including Car, Tif, Lxrβ, Pparα, Ero1β, Reverbα, and Shp. Altered Pparγ induction of target genes provided additional evidence of perturbed hepatic metabolic control elicited by extrahepatic tumors.

**Conclusions:** Extrahepatic malignancy can affect hepatic drug metabolism by nuclear receptor relocalization and decreased receptor expression and function. These findings could aid the design of intervention strategies to normalize drug clearance and metabolic pathways in cancer patients at risk of chemotherapy-induced toxicity or cancer cachexia. *Clin Cancer Res; 17(10); 1–11. ©2011 AACR.*

Introduction

A major challenge to the effective use of cancer chemotherapy is wide interpatient variability in clearance, and consequently, induced side effects of cytotoxic drugs. There is accumulating evidence that the presence of malignancy is accompanied by widespread changes in hepatic gene expression. This is clinically relevant as the liver is responsible for an extensive range of metabolic processes. Clinical studies have also shown that cancer patients with elevated inflammatory markers/symptoms induced by their malignancy have reduced hepatic drug clearance, leading to worse toxicity from anticancer drugs (1–3). In advanced cancer patients, reduced cytochrome P450 3A4 (CYP3A4)–mediated drug metabolism, as indicated by the erythro-
Translational Relevance

The findings provide insight into the mechanisms underlying reduced drug clearance in the setting of cancer and underscore the challenges in therapeutic drug dosing. This could aid the design of intervention strategies to normalize drug clearance and metabolic pathways in cancer patients at risk of chemotherapy-induced toxicity or cancer cachexia.

generally defined as a hypermetabolic wasting disease, which results in progressive depletion of lipid depots and skeletal muscle, irrespective of nutritional intake (10). Cachexia occurs in approximately 50% of cancer patients. However, the incidence of cachexia varies depending on the tumor type, ranging from 70% to 80% in patients with carcinomas of the pancreas and stomach to 8% in patients with cancer of the esophagus (11). Cancer cachexia contributes to morbidity and mortality in these patients, directly accounting for 20%–30% of all cancer deaths (12). As a consequence, cachexia is considered a late event that once established has no effective treatment (13). The mechanisms of CCS are likely to be complex involving cross-talk between cytokine and endocrine signaling pathways with homeostatic regulation of metabolism and energy balance (10, 14).

Nuclear hormone receptors are a superfamily of transcription factors with 48 distinct members identified within the human genome (15). In addition to the classic steroidal hormone receptors, other nuclear receptors act as metabolic sensors that respond to compounds of dietary origin, intermediates in metabolic pathways, drugs, and other environmental factors, integrating homeostatic control over many metabolic processes (16–18). For example, aspects of drug metabolism and transport are regulated by pregnane X receptor (PXR) and constitutive androstane receptor (CAR); energy and glucose metabolism through peroxisome proliferator-activated receptor gamma (PPARY); fatty acid, triglyceride, and lipoprotein metabolism via PPAR alpha (α), delta (δ), and γ; reverse cholesterol transport and cholesterol absorption through liver X receptor (LXR); and bile acid metabolism through farnesoid X receptor (FXR; refs. 17–19). Given that nuclear receptors are central to the regulation of these various metabolic pathways, an understanding of their overall function in tumor-induced metabolic disturbances needs to be developed. Such investigations may aid in understanding the mechanisms underlying metabolic changes, which impact on drug clearance pathways in cancer patients and the dysregulated energy balance that produces cancer cachexia.

In the present study, we employed the Engelbreth-Holm-Swarm (EHS) sarcoma mouse model, a non-metastatic tumor implanted in the quadriceps muscle to investigate the expression and function of hepatic transcription factors and nuclear receptors, particularly in the regulation of drug metabolism involving CYP3A-mediated pathways. The EHS tumor model has been previously shown to be associated with a tumor-mediated inflammatory response, as indicated by increased plasma levels of acute phase proteins and high circulating cytokine concentrations (5, 8, 9). Herein, we show an in vivo tumor-mediated inflammatory model exhibiting impaired action of PXR and CAR in the control of CYP3A expression and more importantly, altered subcellular distribution of their obligatory heterodimerization partner retinoid X receptor alpha (RXRα). Furthermore, we show an extensive effect of extrahepatic tumor on the expression of a number of hepatic nuclear receptors. Thus, the broad perturbations of metabolism observed in cancer patients may be explained by functional impairment of a wide range of hepatic signaling processes mediated by several nuclear receptors and associated with tumor-derived inflammatory stimuli.

Materials and Methods

Tumor mice

All animal experimentation was conducted in accordance with the guidelines of the Australian Council on Animal Care under protocols approved by the Westmead Hospital Animal Ethics Committee. Eight to 10-week-old male FVB mice were aseptically inoculated with 0.3 mL suspension of EHS sarcoma into the right quadriceps muscle using a 16-gauge needle. Control animals were inoculated with the vehicle, Dubelcco’s modified Eagle’s medium (DMEM; GIBCO, Invitrogen) containing penicillin/streptomycin (GIBCO, Invitrogen). At sacrifice, the tumor mass reached approximately 3 g or 10% of total body weight after 2–3 weeks. The liver was immediately harvested, snap frozen in liquid nitrogen, and then stored at −80°C for downstream analysis.

Messenger RNA expression

Total RNA was isolated from frozen mouse liver wedges using Trizol reagent (Invitrogen). Before cDNA synthesis, RNA was treated with DNase I (Ambion) according to the manufacturer’s protocol. cDNA was synthesized from 5 μg of total RNA with SuperScript III cDNA First-Strand Synthesis System, using random hexamer primers and deoxynucleotides. Taqman or SYBR green protocols were used to amplify cDNAs of interest by real-time quantitative PCR (QPCR) using the Rotor-Gene 3000 and 6000 (Corbett Research). mRNA levels were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and 18S ribosomal mRNA expression. Normalization to both housekeeping genes gave comparable results and all genes analyzed are shown with GAPDH normalization. Graphs of mRNA levels are shown as expression relative to a standard curve representing 5-fold dilutions of stock cDNA and are not true concentrations of mRNA abundance. Primers used in these studies were C/ebpα forward GAGCGACGAGTACAAGA, reverse GCCAGCTCCAGCAG-
by specific antibodies were visualized using a SuperSignal against Coomassie stained protein bands. Proteins detected at 42 kDa could be visualized simultaneously and the normalizing protein, -Actin or normalized to GAPDH, were calculated using the comparative delta-Ct method and a 7900HT sequence detection system. Relative mRNA levels were assessed using Superscript II reverse transcriptase (Invitrogen). Primers and probes were designed using ABI PrimerExpress software for use in the NIH-funded Nuclear Receptor Signaling Atlas Project (NURSA) and were subjected to extensive validation. Sequences of primers and probes are available on the (www.NURSA.org) website. High-throughput processing technique. Briefly, cDNA was synthesized from 2 μg of DNase-treated total RNA using Superscript II reverse transcriptase (Invitrogen). Primers and probes were designed using ABI PrimerExpress software for use in the NIH-funded Nuclear Receptor Signaling Atlas Project (NURSA) and were subjected to extensive validation. Sequences of primers and probes are available on the (www.NURSA.org) website. High-throughput processing was achieved using a semiautomated Beckman liquid handler, followed by an ABI Prism 7900HT sequence detection system. Relative mRNA levels were calculated using the comparative delta-Ct method and normalized against both GAPDH and U36b4 mRNA levels in the same total RNA samples. "Both housekeepers gave comparable results and only GAPDH normalized data are available on the (www.NURSA.org) website."

Western blot analysis

Extraction and preparation of proteins from liver tissue were carried out as previously described (20). In brief, 50 mg of liver tissue was homogenized in ERK Buffer (50 mmol/L HEPES, 150 mmol/L NaCl, 1.5 mmol/L MgCl2, 1 mmol/L EGTA, 10% glycerol, and 0.1% TritonX-100) containing a mix of protease inhibitors (PMSF, DTT, leupeptin, aprotonin, sodium fluoride, and sodium orthovanadate). Protein concentrations for equal loading were determined using the Bio-Rad DC assay kit (Hercules). Total RNA extracted from livers of control and EHS tumor-bearing mice, as described above, were profiled for nuclear receptor expression at the Gene Expression Laboratory, Salk Institute, using a real-time PCR–based high-throughput processing technique. Briefly, cDNA was synthesized from 2 μg of DNase-treated total RNA using Superscript II reverse transcriptase (Invitrogen). Primers and probes were designed using ABI PrimerExpress software for use in the NIH-funded Nuclear Receptor Signaling Atlas Project (NURSA) and were subjected to extensive validation. Sequences of primers and probes are available on the (www.NURSA.org) website. High-throughput processing was achieved using a semiautomated Beckman liquid handler, followed by an ABI Prism 7900HT sequence detection system. Relative mRNA levels were calculated using the comparative delta-Ct method and normalized against both GAPDH and U36b4 mRNA levels in the same total RNA samples. "Both housekeepers gave comparable results and only GAPDH normalized data are available on the (www.NURSA.org) website."

Immunofluorescent detection of RXRα

Paraffin-fixed liver wedges from control and tumor-bearing mice were cut on a microtome (Leica RM2121RT), 3 mm thick and mounted onto Superfrost Plus slides (Menzel-Glaser). Following paraffin removal, tissues were permeabilized with PBS (0.1%) Triton X-100 for 15 minutes, washed, and incubated with the following: Image-iT FX signal enhancer (Invitrogen; catalogue no. I36933) for 30 minutes, Background Buster (Innovex Biosciences; catalogue no. NB306) for 10 minutes, and Streptavidin/AlexaFluor 555 (Invitrogen, Molecular Sciences; catalogue no. SB04) at 1:800 dilution and then for another 15 minutes, washed, and incubated with the following; 1:1,000 dilution of anti-rabbit RXRα antibody (Santa Cruz Biotechnology; catalogue no. sc-553) in a humidified chamber at 4°C. Following PBST washes, slides were incubated for 30 minutes with anti-rabbit secondary antibody (ABCAM, Sapphire Biosciences; catalogue no. A66012) at 1:800 dilution and then for another 30 minutes with Streptavidin/AlexaFluor 555 (Invitrogen, Molecular Probes; catalogue no. S32355) at 1:1,000 dilution, light protected. Nuclei staining was conducted using DAPI (Invitrogen; catalogue no. D21490). Slides were coverslipped using Prolong Gold antifade reagent (Invitrogen, Molecular Probes; catalogue no. P36934) and visualized with a Leica BMBL upright microscope and Spot Advanced version 4.1 software (Diagnostic Instruments, Sterling Heights). Negative controls followed all outlined procedures except RXRα antibody treatment.

Functional assessment of CAR and PXR

Ten to 12-week-old male FVB mice hemizygous for the ~13kb CYP3A4/Δα transgene (21), with or without EHS tumor, were administered single daily i.p. injections of pregnenolone-16α-carbonitrile (PCN; 40 mg/kg/d) or 1,4-Bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP; 1 mg/kg/d) over 3 days. Control mice received the ligand vehicle corn oil. Ligand injections were conducted after 2–3 weeks of tumor growth and 3 days before the due harvest date. PCN was purchased from MP Biomedicals, Inc., TCPOBOP from Maybridge Chemical Company. CAR- and PXR-induced CYP3A4 transgene expression in liver wedges were macroscopically detected and quantified using densitometric analysis.

Nuclear and cytoplasmic extract preparations

Preparation of nuclear and cytoplasmic protein extracts was made using the ProteoExtract Subcellular Proteome Extraction Kit (Calbiochem, MERCK; catalogue no. 539790) as per the manufacturer’s instructions. Fifty milligrams of frozen liver tissue was homogenized by 2–4 passes using a plastic pestle fit for a 1.5 mL microcentrifuge tube. All buffers were provided in the kit and all procedures were conducted on ice.
using X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) staining, (Astral Pty. Ltd.) and ONPG (O-nitrophenyl-β-D-galactopyranoside) assays, (Sigma–Aldrich), respectively. These procedures have been previously described (21).

**Functional assessment of PPARα and PPARγ**

Tumor-bearing and nontumor male FVB mice were injected with PPARα and PPARγ receptor specific agonists Wy-14643 (Saphire Biosciences, Cayman Chemicals; catalogue no. 190-70820) at 100 mg/kg/d and troglitazone (Cayman Chemical; catalogue no. 71750) at 150 mg/kg/d for 3 days before harvest. Ligand doses were chosen on the basis of existing literature (22–24). Control animals were administered 100 μL of the vehicle consisting of 1.5% carboxymethylcellulose (CMC) and 0.2% Tween 20 in sterile water. Hepatic PPARα and PPARγ activities in presence of tumor were assessed by analyzing the mRNA level of target gene induction following ligand activation using real-time QPCR. Target genes assessed for PPARα included Cyp4a14 forward GACGCTCCATACCCA, reverse GCCGCAAACGTGGGT, Hmg-CoA reductase forward CTTGGCATTGCCT, reverse AGCCGAACGACATGATG, Cpt1α, forward CTCTACTCTCGCCATCC, reverse CAGCTCTCTTCACGTGGT, and for PPARγ included, Lpl forward GAGGTCCTCCTAAGGGA, reverse TGGACGTTGTCTAGGGGGTA and Cyp3a, forward TTGTATACACTTTT, reverse CTGGTTTTTG-AACATTTCGT.

**Data analysis and statistics**

Quantitative data were expressed as mean ± SEM. Statistical analyses between control and tumor groups were conducted using the unpaired Student’s t-test. Significance was established at \( P \leq 0.05 \).

**Results**

**The EHS tumor mouse model**

The EHS tumor is a transplantable mouse xenograft tumor that spontaneously arose in a ST/Eh strain mouse (25). The expression profile of EHS using CDNA microarrays has identified the tumor as derived from the parietal endoderm (26) and the tumor itself has been used widely as a cell culture substrate that mimics an extracellular matrix. Once implanted, the EHS tumors were grown for 2–3 weeks such that excessive tumor burden injurious to general animal health was avoided. These tumor-bearing mice have been previously reported to exhibit reduced drug metabolism with decreased CYP3A-mediated enzyme activity. Furthermore, decreased CYP3A enzyme activity was shown to correlate with reduced hepatic Cyp3a protein and mRNA expression, encompassing both the humanized CYP3A4 reporter transgene and its endogenous mouse homologue, Cyp3a11 (8, 9). Thus, Cyp3a mRNA levels are a suitable surrogate of CYP3A-mediated metabolism. In the present study, the EHS mice exhibited similar decreased Cyp3a expression.

**Impact of cancer on constitutive regulators of CYP3A**

Decreased mRNA expression of CYP3A suggested the EHS tumor affects transcription factors responsible for their regulation in the liver. The impact of malignancy on major constitutive CYP3A regulators showed no statistically significant changes in mRNA for HNF4α, CCAAT enhancer-binding protein (C/EBP)β (Fig. 1A); or C/EBPα, HNF3γ,
and albumin D-site binding protein (DBP; data not shown) in tumor-bearing mice as compared with controls. Western blot analysis showed changes in total C/EBPβ protein, whereas HNF4α protein levels were not altered between the control and tumor groups (Fig. 1B). C/EBPβ has a number of isoforms including C/EBPβ liver activating protein (LAP) and C/EBPβ liver inhibitory protein (LIP). These isoforms have different roles in the regulation of CYP3A genes (27) and changes in the LIP/LAP ratio have been shown to be responsible for the IL-6-mediated repression of CYP3A4 in hepatic and nonhepatic cultured cells (28). In our in vivo tumor model, there was no difference in the LIP/LAP ratio to explain a similar mechanism of basal CYP3A repression. As determined by densitometric analysis of western blots, both isoforms were decreased equally in the presence of tumor (Fig. 1B (i)). Nonetheless, significant repression of both C/EBPβ isoforms in tumor-bearing mouse livers may potentially impact on CYP3A basal levels.

**Tumor-bearing mice exhibit impaired PXR and CAR function**

The predominant inductive transcriptional regulators of CYP3A genes are the nuclear receptors PXR and CAR. Once activated by their ligands, these receptors heterodimerize with RXRα and bind to cis-acting elements in CYP3A genes to enhance transcription. Using real-time PCR analysis, tumor-bearing animals showed a significant decrease in CAR expression and a trend toward PXR and RXRα repression that did not attain statistical significance (data not shown, see Fig. 5 for a summary of profiled NR expression). To investigate the impact of tumor growth on hepatic PXR and CAR, their functional activity in the presence of the EHS sarcoma was examined. Activation of PXR and CAR was achieved by administration of PCN and TCPOBOP, respectively, and these agonists were used to determine the integrity of PXR- and CAR-mediated CYP3A induction. In addition, mice incorporating a ~13 kb CYP3A4/lacZ regulatory transgene were employed providing a direct readout of the function of the human CYP3A4 gene promoter in vivo.

Confirming our previous findings, X-Gal staining of liver wedges without ligand treatment showed reduced basal transcription of the CYP3A4 transgene in tumor-bearing mice (Fig. 2A and 3A; ref. 8). Following PXR and CAR activation by PCN and TCPOBOP, respectively, control mice exhibited substantial CYP3A4 induction as determined by both the X-Gal staining and the ONPG assays, whereas induction by both PCN and TCPOBOP was significantly abrogated in the tumor-bearing cohort (Fig. 2A, 2B and Fig. 3A, 3B). Similarly, endogenous mouse hepatic Cyp3a11 and Cyp2b10 mRNA levels were induced by TCPOBOP in the controls with a significantly lower induction in tumor-bearing mice (Fig. 3C and 3D). Following PCN treatment, the apparent induction of the endogenous mouse Cyp3a11 gene exhibited a trend toward a decreased degree of induction.
in the tumor mice. However, no statistical significance was reached when compared with the induction potential of activated PXR in the control animals (Fig. 2C).

Cytoplasmic accrual of RXRα protein in tumor mice

Following an acute inflammatory response, hepatic RXRα protein has been reported to undergo cytoplasmic relocalization, leading to decreased nuclear RXRα levels (29, 30). To investigate whether the presence of extra-hepatic tumor has similar effects, RXRα localization was examined by Western blot and immunofluorescence in liver sections. Total cellular content of RXRα protein was found to be equivalent between the groups (Fig. 4A). However, nuclear abundance of RXRα in the livers of tumor-bearing mice was substantially decreased, whereas in the cytoplasmic fraction was increased relative to controls (Fig. 4B). To confirm the apparent cytoplasmic retention of RXRα in tumor-bearing mice, immunofluorescence staining was carried out on liver sections (Fig. 4C). In control animals, RXRα was clearly localized predominantly in the nucleus within hepatocytes. In tumor-bearing mice, most RXRα was retained in the cytoplasm.

Impact of tumor on hepatic nuclear receptor superfamily

Nuclear receptors are pivotal regulators of many metabolic processes, including energy homeostasis and drug metabolism (17–19). Livers from control mice and mice bearing the EHS tumor were profiled at the mRNA level for all 49 murine nuclear receptor superfamily members using high-throughput real-time QPCR. Sixteen out of the 40 nuclear receptors expressed in the liver showed significant differential expression in tumor-bearing animals (Fig. 5A).

Interestingly, with the exception of HNF4γ and VDR, all changes in nuclear receptor levels in the presence of extrahepatic tumor revealed decreased expression. Of these changes, when broadly categorized on the basis of receptor’s physiological ligands and potential functions, 5 nuclear receptors belonged to the endocrine receptor family, 4 belonged to the adopted orphan receptor family, and 7 to the true orphan nuclear receptors (Fig. 5B).

Nuclear receptors, PPARα and PPARγ, which are predominantly involved in lipid and carbohydrate homeostasis were further examined at the functional level. Administration of PPARα and PPARγ receptor specific ligands, Wy-14643 and troglitazone, respectively, showed evidence of repressed receptor function in presence of extrahepatic...
tumor (Fig. 6). Well-characterized PPARα and PPARγ target genes examined all showed significant induction by ligand treatment in control nontumor bearing mice. The response was reduced for the PPARα target genes, Hmg-CoA and Cpt1α in tumor mice, whereas Cyp4a14 was robustly induced (Fig. 6A). The induction of PPARγ target genes also showed a mixed response in tumor mice, with Cd36 exhibiting impaired induction, whereas no change in induction of Lpl was observed (Fig. 6B). Evidence of changes in PPARα and PPARγ target gene expression in tumor mice provide supportive evidence of disturbed hepatic function, particularly related to lipid and glucose metabolism.

Discussion

These studies show that profound changes in hepatic drug clearance in tumor-bearing mice can be due to broad suppression of the transcriptional regulators of genes encoding drug clearance proteins such as PXR and CAR.

Figure 4. Altered nuclear and cytoplasmic distribution of liver RXRα protein in presence of extrahepatic tumor. A, Western blot analysis of total hepatic RXRα protein in control (n = 7) and tumor-bearing (n = 8) groups. A representative Western blot of RXRα normalized against β-Actin is shown together with the densitometric measure of protein band quantification. B, Western blot analysis of nuclear and cytoplasmic liver protein extracts for RXRα localization in livers of control versus EHS tumor animals. Representative blots with 50 µg of nuclear or cytoplasmic proteins in each lane are shown with corresponding Coomassie stains of each membrane to confirm equal protein loading. C, immunofluorescent imaging of RXRα (i–iii), DAPI nuclear staining (iv–vi), and an overlay image of both RXRα fluorescence and DAPI (vii–ix) in hepatocytes of control mice (top panel) and tumor mice (second panel). Bottom panel shows a negative control lacking RXRα antibody. Images are captured at ×40 objective.
associated with a broad range of disease states can repress those associated with the CCS. Perturbations in metabolic pathways and energy balance relative changes may underlie more general hepatic functions, and perhaps more importantly to a concomitant change with 13 other nuclear receptors, the resulting cumulative changes with as well as the major regulators of drug metabolism, PXR, CAR, and their dimerization partner RXRα (31–33). Such repression can lead to profound changes in the expression of important drug metabolizing enzymes, such as CYP3A4, also known to be altered under diverse pathological conditions (34, 35). However, studies that examine the mechanistic link between disease and decreased expression of drug metabolizing enzymes have commonly employed LPS, turpentine, or direct administration of cytokines to elicit or mimic acute inflammatory states. Little information is available regarding alterations in transcription factors, nuclear receptors, and important drug metabolizing enzymes in complex disease settings involving a chronic inflammatory response, such as is often observed in cancer patients. Thus, these studies provide the first mechanistic information concerning CYP3A repression using an in vivo cancer model. We show only significantly decreased CAR mRNA levels in tumor-bearing mice as opposed to the broad transcriptional repression of many transcription factors under acute inflammatory conditions. However, the function of both CAR and PXR was impaired, as determined by CYP3A4 regulatory transgene induction in the presence of the EHS tumor. In tumor-bearing mice, both PXR and CAR ligands failed to induce CYP3A4 transgene expression to the same extent as in control animals. Functional CAR impairment was further confirmed by the reduced degree of induction of mouse Cyp3a11 and Cyp2b10 expression in response to TCPOBOP. The observation that the decrease in PXR-mediated Cyp3a11 induction in tumor mice was not as great as that seen with the CYP3A4 transgene could be due to species-specific differences in PXR DNA-binding elements between mouse Cyp3a11 and human CYP3A4. To date, no transcriptional enhancer equivalent to the human CYP3A4 xenobiocresponsive element (XREM; ref. 36) has been identified in the mouse Cyp3a gene cluster. Nonetheless, evidence of impaired CAR and PXR function may provide a partial explanation for repression of CYP3A4-mediated metabolism. Furthermore, their functional impairment could also potentially impact on a number of important drug metabolizing and disposition enzymes as well as contributing to perturbed energy balance (37).

This is linked to a reduction in their expression, impaired function, and perhaps more importantly to a concomitant cytoplasmic accumulation of RXRα. Because RXRα interacts with 13 other nuclear receptors, the resulting cumulative changes may underlie more general hepatic perturbations in metabolic pathways and energy balance that are associated with the CCS.

It has been recognized that inflammatory mediators associated with a broad range of disease states can repress hepatic transcription factors such as C/EBPα and HNF4α (31) as well as the major regulators of drug metabolism, LXRα, PPARγ, RXRα, RARα, Fxr, PXR, GR, TRα, LXRα, TR2/4, RORα/γ, Cyp4a14, Hmg-CoA, SREBP, TRβ1/2, Shp, GCNF, PGC1α, GCNF, RXRα/γ.

Figure 5. Differential expression of nuclear receptors in livers of mice bearing extra-hepatic EHS tumor. A, pie chart showing the expression fold change of all detected nuclear receptors expressed in livers of tumor-bearing mice as compared with control mice. Varying fold changes are denoted as different colored segments with green representing downregulation and red representing upregulation. B, a tabular listing of altered nuclear receptor expression under the broadly classified physiological functional subgroups. Five endocrine, 4 adopted orphan, and 7 true orphan receptors exhibit altered mRNA expression. Fold changes with $P \leq 0.05$ were considered significant ($n = 5$ per group).

Figure 6. Activity of hepatic PPARα and PPARγ in presence of extrahepatic EHS tumors. Basal and induced target gene expression for PPARα (A) and PPARγ (B). Induced gene expression in nontumor controls and tumor-bearing mice as achieved by treatment with Wy-14,643 (+Wy) for PPARα activation and troglitazone (+Trog) for PPARγ activation ($n = 6–9$ per group). $^*$, $P < 0.05$ denotes a significant change in gene expression in control versus tumor groups following ligand activation.
Decreased nuclear and increased cytoplasmic RXRα seen with Western blot analysis and immunofluorescence suggest that in the presence of cancer the activity of RXRα is decreased. RXRα is the obligate heterodimerization partner of class II nuclear receptors, such as PXR, CAR, VDR, PPARs, FXR, RAR, TR, and LXR (18). Therefore, cytoplasmic retention of RXRα may contribute to the functional impairment of PXR and CAR seen in tumor-bearing animals. Furthermore, as the obligate heterodimerization partner of class II NRs, decreased nuclear availability of RXRα widens the scope of tumor-mediated perturbations in the liver, beyond drug metabolism. Reduced nuclear availability of RXRα, which has been previously showed only in acute inflammation (29, 38) suggests that similar pathways could also be operative in the presence of cancer exhibiting a chronic inflammatory phenotype. Thus, tumor-mediated inflammatory signaling in the liver is likely to influence nuclear receptor function, resulting in dysregulated metabolic processes. The role of specific cytokines in this process could be explored with blocking antibodies or other interventions to disrupt downstream signaling pathways. Such an approach would distinguish between direct effects of cytokines from compensatory changes in overall metabolic balance associated with tumor growth.

The potential for the hepatic expression of nuclear receptors to be altered by extrahepatic cancer has not been previously considered. In the present study, all 49 mouse nuclear receptors were profiled in an attempt to gain a better understanding of affected metabolic pathways. Tumor effects were observed among 16 endocrine, adopted orphan, and orphan receptors, with the majority of affected nuclear receptors showing decreased expression. Changes in endocrine nuclear receptors can have complex and profound effects on physiology and energy metabolism. Altered MR, TRβ, RARα, AR, and VDR seen in tumor-bearing animals implies alterations in electrolyte and fluid balance, metabolic rate and oxidative metabolism, cell physiology, reproductive function, and general homeostasis (39–42). Repression of orphan nuclear receptors such as Reverb α and β, which have a diverse function in regulating cell physiology and circadian rhythm (43) indicates broad tumor-related disturbances in hepatic physiology. Decreased expression of PPARα, LXRβ, and CAR in tumor-bearing mice translates into disturbed regulation of fatty acid oxidation, cholesterol homeostasis and, as discussed above, xenobiotic metabolism. Impaired CAR action may also contribute to perturbed energy balance as it has been shown to play a role in adaptation to metabolic stress (37). Functional assessment of hepatic PPARα and PPARγ showed some impairment of their action for selected but not all target genes and provided further evidence of tumor effects on nuclear receptor activity. It would be interesting to carry out expression profiling by microarray analysis of livers from tumor-bearing mice to characterize the impact of altered nuclear receptors on hepatic metabolism in cancer.

Ligands that modulate nuclear receptor activity have significant potential in therapeutic applications. From our studies, we can speculate that enhancing the activation of the nuclear receptors PXR or CAR prior to chemotherapy may ameliorate toxicity in those patients showing poor drug metabolism. It is even more appealing to speculate that targeted therapies focused on RXR function may provide novel means of restoring not only pathways in drug metabolism but also other vital hepatic functions regulated by its essential binding. RXR is activated by its endogenous ligand 9-cis retinoic acid (44) and several RXR-selective agonists known as "rexinoids" have been developed (45, 46). It has yet to be determined if rexinoid treatment will result in increased nuclear availability in tumor mouse hepatocytes to allow heterodimerization with other class II nuclear receptors.

In summary, our findings suggest that extrahepatic tumors can decrease transcriptional expression of hepatic CYP3A genes in part by reductions in C/EBPα protein and impaired function of PXR and CAR. Furthermore, our results suggest that decreased nuclear availability of RXRα may explain impaired activity of both CAR and PXR and lead to functional impairment of other nuclear receptor-regulated pathways that rely on RXRα heterodimerization. Thus, altered hepatic nuclear receptor function may be one mechanism underlying tumor-mediated cancer cachexia, which involves a complex array of perturbed metabolic functions. With a better understanding of the mechanistic links between extrahepatic tumors and impaired nuclear receptor action in the liver, therapies based on inhibiting or stimulating specific nuclear receptors represents a promising intervention approach to potentially reduce aberrant toxic side effects associated with anticancer treatments and possibly aid in the prevention of metabolic abnormalities that lead to cancer cachexia. Although this study has focused on such processes in the context of cancer, the findings of altered basal transcription factors and impaired hepatic nuclear receptor action may be relevant to many other clinical settings involving chronic inflammation and cachexia.

Disclosure of Potential Conflicts of Interest

The authors have no conflicts of interest to declare.

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