The Aryl Hydrocarbon Receptor Functions as a Tumor Suppressor of Liver Carcinogenesis

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Abstract

The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that mediates the biological and toxic effects of its xenobiotic ligands. Previous cell culture studies have shown that, in addition to controlling the xenobiotic detoxification response, AHR activation leads to G0-G1 arrest, diminished capacity for DNA replication, and inhibition of cell proliferation. In fact, recent work from our own and from other laboratories suggests that AHR may function as a tumor suppressor gene that becomes silenced during the process of tumor formation. To test this hypothesis and determine whether the mouse Ahr gene acts as a tumor suppressor gene in vivo, we have examined the role of Ahr ablation in liver tumorigenesis induced by the genotoxic chemical diethylnitrosamine (DEN), a hepatic carcinogen that is not an AHR ligand. In mice given a single i.p. injection of DEN, AHR antagonized liver tumor formation and growth by regulating cell proliferation, inflammatory cytokine expression, and DNA damage, parameters which were significantly elevated in the livers of control and, more so, of DEN-exposed Ahr−/− mice. Ahr−/− hepatocytes also showed significantly higher numbers of 4N cells, increased expression of proliferative markers, and repression of tumor suppressor genes. These data support the concept that in its basal state in the absence of a xenobiotic ligand, the Ahr gene functions as a tumor suppressor gene, and that its silencing may be associated with cancer progression. Cancer Res 70(1): 212–20. ©2010 AACR.

Introduction

The aryl hydrocarbon receptor (AHR), a ligand-activated member of the basic-region helix-loop-helix/period-aryl hydrocarbon nuclear translocator-simple-minded (bHLH/PAS) family of transcription factors, controls a variety of developmental and physiologic events, including induction of drug metabolizing enzymes, xenobiotic detoxification, neurogenesis, tracheal and salivary duct formation, circadian rhythms, response to hypoxia, and hormone receptor function (1–3). More than 400 environmental toxicants and naturally occurring compounds are reported to bind and activate this cytosolic receptor (4) to form a nuclear heterodimeric transcription factor through dimerization with aryl hydrocarbon receptor nuclear translocator (ARNT), also a bHLH/PAS protein. The transcriptionally active AHR/ARNT heterodimer recruits the basal transcriptional machinery, remodels chromatin, and initiates transcription of genes coding for many phase I drug metabolizing enzymes, such as the cytochromes P450 CYP1A1, CYP1A2 and CYP1B1 and several phase II conjugating enzymes such as aldehyde dehydrogenase 3A1 and NAPDH-dependent quinone oxidoreductase (5–7). Following nuclear export, AHR is degraded via the 26S proteasome pathway (8).

The AHR also functions as a cell cycle regulator (9–13) by promoting cell cycle progression in the absence of an exogenous ligand (14–16), by promoting growth and immortalization of keratinocytes (17), or by inhibiting cell proliferation in cells exposed to the prototypical ligand, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD; refs. 18–21). From a mechanistic viewpoint, at least two different signaling pathways contribute to the role of AHR in cell cycle regulation. On one hand, AHR blocks transforming growth factor β1 (TGFβ1)-dependent inhibition of cell proliferation and promotion of apoptosis by repressing TGFβ1 expression (15, 22) and by accelerating TGFβ1 mRNA degradation (16). In addition, fibroblasts from AHR-knockout mice overproduce TGFβ1 (15, 16) and their livers overproduce TGFβ1 and TGFβ3 (23), causing low proliferation rates and increased apoptosis. On the other hand, protein interactions between AHR and the retinoblastoma protein (RB)/E2F axis further repress S-phase gene expression and prevent entry of the cells into S phase (10, 11, 13, 20). Hence, depending on the cellular environment, Ahr could be considered a pro-proliferative gene in some cases and an antiproliferative gene in others. Consistent with at least one aspect of this view, there is increasing evidence that AHR may function as a tumor suppressor in vivo. Epigenetic Ahr silencing by promoter
hypermethylation has recently been reported in a significant number of human acute lymphoblastic leukemia cases (24). Ahr repression was also substantial in diethylnitrosamine (DEN)-induced liver tumors of mice, albeit in the absence of any significant promoter hypermethylation (25). Furthermore, AHR-null transgenic adenocarcinoma of the mouse prostate (TRAMP) mice show increased susceptibility to prostate tumorigenesis and develop prostate tumors with greater frequency than Ahr+/− TRAMP mice, suggesting that AHR possesses tumor suppressor properties (26).

To study the role of the Ahr gene in liver tumorigenesis, we have examined tumor incidence in DEN-induced liver tumors in wild-type and Ahr−/− mice. DEN is metabolized by the cytochrome P450 CYP2E1 into a carcinogenic alkylating agent that causes DNA damage, but DEN is not an AHR ligand nor does it induce AHR responsive detoxification genes (27, 28). Using DEN allowed us to explore AHR functions that do not depend on xenobiotic AHR ligands or that result from AHR-mediated procarcinogen activation. The central question in these experiments was to determine whether loss of Ahr affected the formation and progression of liver tumors in DEN-induced mice and, if it did, to characterize the molecular changes associated with the process. Our data are consistent with the hypothesis that during DEN-induced carcinogenesis, Ahr functions as a tumor suppressor gene.

Materials and Methods

Animals and hepatocarcinogen treatment. The Ahr−/− mice used in these experiments were derived from Ahr−/− mice bred into a C57BL/6j genetic background at The Jackson Laboratory. These mice were purchased 6 y ago and were thereafter maintained in our colony. Ahr−/− mice were bred with wild-type Ahr+/− mice and maintained as hemizygous Ahr−/− mice housed in a pathogen-free animal facility under a standard 12-h light/12-h dark cycle with ad libitum water and chow. All experiments were conducted using the highest standards of human care in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the University of Cincinnati Institutional Animal Care and Use Committee. For experimental treatment, sibling hemizygous mice were bred and the litters genotyped for loss or presence of the wild-type allele in homozygosis. Groups (n = 5–9) of 15-d-old Ahr+/+ and Ahr−/− male and female littermates were administered a single i.p. injection of the genotoxic hepatocarcinogen DEN (Sigma) dissolved in saline at a dose of 20 mg/kg body weight. Littermates used as controls were injected with an equal volume of saline. Mice were sacrificed at 3 to 4 wk or at 30 to 35 wk after DEN injection. To measure cell proliferation rates, mice were given a single i.p. injection of 150 mg/kg bromodeoxyuridine (BrdUrd) 90 min before euthanasia. Immediately after euthanasia, livers were excised, weighed, and photographed to facilitate scoring of surface liver lesions. One lobe was rapidly fixed in buffered 10% formalin for 16 h for histologic analysis. Visible surface tumors were excised from both Ahr+/+ and Ahr−/− animals, immediately frozen in liquid N2, and stored at −80°C until use.

Tumor quantification. Grossly visible surface liver tumors were scored with the aid of digital photographs of the top and bottom of each liver. The size of surface nodules was quantified by measuring the diameter of nodules in millimeters. Liver histology and pathology were evaluated by microscopic evaluation of H&E-stained sections in a blinded fashion by a veterinary pathologist (G.P.B.).

Preparation of liver tissues for histology, BrdUrd staining analyses, γH2AX assays, and ploidy determination. Formalin-fixed, paraffin-embedded liver tissues were sectioned at 4 μm. For immunohistochemical staining, sections were deparaffinized in xylene and rehydrated through a graded series of ethanol/water solutions. For histology evaluation, H&E staining was done by standard protocols. BrdUrd incorporation was analyzed using a streptavidin-biotin BrdUrd detection kit (Zymed) exactly as recommended by the manufacturer. BrdUrd incorporation was scored blind, and 400 to 700 hepatocytes per field were counted from six to eight random sections from each mouse liver; the mean ± SEM of at least three mice in each group was recorded. For γH2AX immunostaining, sections were boiled for 10 min in antigen retrieval solution [10 mmol/L sodium citrate sodium buffer (pH 6.0); DakoCytomation] and cooled to room temperature before proceeding with staining. Endogenous peroxidase activity was quenched by treatment with 3% H2O2 in methanol. Sections were blocked in 0.25% normal goat serum in TBS for 1 h at room temperature and incubated for 1 h at 37°C with monoclonal antibodies to histone γH2AX (γH2AX; JBW301 from Millipore) at 1:1,500 dilution. Normal mouse serum was used as negative control. Sections were incubated for 1 h at room temperature with biotinylated goat anti-mouse secondary antibody (anti-mouse IgG staining systems, Santa Cruz Biotechnology) at 1:200 dilution, counterstained with hematoxylin, dehydrated, mounted in permount medium, and visualized under a light microscope. Positive cells were scored as per the BrdUrd staining protocol. Flow cytometric determination of γH2AX was done as previously described (13) by incubation with FITC-conjugated anti-phosphohistone H2A.X (p-Ser139) or negative control mouse IgG-FITC. Determination of ploidy levels was also as previously described by one of us (29).

Cytokine determination. Liver homogenates in PBS supplemented with a cocktail of protease inhibitors (Roche) were centrifuged at 2,500 x g to clarify the lysates and used to determine the levels of the two cytokines, interleukin-6 (IL-6) and tumor necrosis factor-α (TNFα), using the Aushon Biosystems Searchlight ELISA services.

RNA extraction, cDNA preparation, and real-time PCR. RNA was isolated from at least three frozen DEN-induced liver tumors and three control livers of each genotype using Tri-reagent (Molecular Research Center, Inc.). Because tumor incidence was low in females, only livers from males were used for these experiments. cDNA was synthesized by reverse transcription of 30 μg of total RNA in a total volume of 45 μL containing 1× reverse transcriptase buffer, 7 μmol/L random hexamers primer, 0.5 mmol/L deoxynucleotide triphosphate mix, 10 mmol/L DTT, 5 mmol/L MgCl2, 20 units of RNase inhibitor (RNasin, Promega), and 100 units of SuperScript II

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were quantified using threshold cycle (C\text{t}) values. C\text{t} values for each mouse sample were determined in duplicate and then normalized to values for β-actin amplification of the same sample (ΔC\text{t}), and the mean of three mice was used to calculate the log 2 of the ratio of the means ± SEM of each gene in the different Ahr−/− tissues (control, tumor, or tumor-adjacent normal) relative to their expression in the corresponding tissues of Ahr+/+ mice. Amplification of β-actin cDNA in the same samples was used as an internal control for all real-time PCR amplification reactions. PCR product specificity was confirmed using melting curve analyses and subsequent PAGE. Primers for cDNA amplification are shown in Supplementary Table S1.

Statistical analyses. Statistical analyses of reverse transcription-PCR (RT-PCR) data were done using SigmaStat 3.1. Tumor incidence comparisons were made using χ² analysis. Group comparisons were made using two-tailed Student’s t test. P < 0.05 was considered statistically significant.

Results

Loss of Ahr promotes DEN-induced tumor formation. In the mouse liver, AHR is a master regulator of the detoxification of xenobiotic polycyclic aromatic hydrocarbons (30) and, through cooperative interactions with the RB/E2F axis, serves as an environmental checkpoint in the regulation of cell cycle progression (31). To investigate whether AHR plays a role in liver tumorigenesis, we analyzed the susceptibility of mice with a deletion of the Ahr gene to develop liver tumors after a single injection of the liver-specific carcinogen DEN. We found a significant enhancement of tumor incidence in the livers of Ahr−/− male mice compared with that in wild-type littermates of the same gender, characterized by significantly increased incidence, multiplicity of nodules, and size (Table 1). Specifically, the number of nodules per mouse was 4-fold higher and the nodule size was double in Ahr−/− mice compared with Ahr+/+ mice. As is the case of liver cancer in humans, females showed fewer tumors than males, in agreement with previous observations in DEN-induced mouse liver cancer (32). Histologic analysis of livers confirmed these findings. Liver nodules typically consisted of basophilic lesions with crowded nuclei and were classified as atypical foci, dysplastic adenomas, or hepatocellular adenomas, characterized by clearly defined margins and compression of surrounding parenchyma. These lesions are believed to be precursors of hepatocellular carcinoma (HCC) in humans (33). One single tumor in a DEN-exposed male presented with disorganized architecture and evidence of necrosis and was classified as HCC. These data support the idea that loss of the Ahr gene can be a critical event in liver cancer progression.
Ahr ablation deregulates proliferation and enhances DNA damage and apoptosis after DEN treatment. In previous cell culture work, we found that loss of Ahr in embryonic fibroblasts of Ahr<sup>−/−</sup> mice leads to a decrease in proliferation rate (16) and an increase in oxidative stress, DNA damage, and apoptosis (13). To determine what role AHR deficiency in the liver might play in tumor development, we used immunohistochemistry to examine livers from control and DEN-induced tumors in male mice of both genotypes. After 30 to 35 weeks of DEN treatment, less than 0.5% of the hepatocytes from unexposed mice of either genotype incorporated BrdUrd, whereas incorporation in livers from DEN-treated mice was significantly higher in Ahr<sup>−/−</sup> than in Ahr<sup>+/+</sup> livers. BrdUrd staining was mainly circumscribed to the tumor, with little or no staining in the nontumor areas. In contrast, Ahr<sup>−/−</sup> hepatocytes from livers of control untreated mice already showed a significant difference in phosphorylated H2A.X histone immunostaining from their Ahr<sup>+/+</sup> counterparts, a difference that was even greater in livers from DEN-treated mice. Terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) analyses revealed no difference in the level of spontaneous apoptosis in untreated mice, but a large difference in DEN-induced apoptosis, with Ahr<sup>−/−</sup> livers showing 10-fold more apoptotic cells than Ahr<sup>+/+</sup> livers. The histologic and immunohistochemistry data are shown in Fig. 1A and B and graphically summarized in Fig. 1C. Overall, the liver data on γH2A.X and TUNEL staining are consistent with our earlier cell culture findings, and show that even in the untreated mice, the Ahr<sup>−/−</sup> genotype confers susceptibility to DNA damage and is proapoptotic. Unlike in cultured cells, Ahr ablation does not deregulate proliferation, which is consistent with the fact that hepatocytes are quiescent in the liver of the intact animal (33).

AHR deficiency impairs ploidy control. Genomic instability is intimately connected with the control of ploidy, with important implications for the pathophysiology of cancer (34). Oftentimes, exposure of cells in culture to genotoxic agents results in the acquisition of abnormal ploidy (35, 36). To assess the effect of DEN exposure on ploidy levels in AHR-proficient and AHR-deficient livers, we dissected tissues from livers of untreated Ahr<sup>+/+</sup> and Ahr<sup>−/−</sup> mice and from liver tumors and normal surrounding tissues of DEN-exposed mice.
of both genotypes. We used flow cytometry to analyze the DNA content of individual nuclei from these tissues. Compared with untreated Ahr−/− animals, exposure to DEN caused no changes in the ratios of 4N:2N hepatocytes, with approximately twice as many 2N than 4N hepatocytes in tumor or surrounding normal liver tissue of the same mice. In contrast, DEN induced a significant increase in the 4N:2N ratios in Ahr−/− mice, with approximately equal numbers of 4N and 2N hepatocytes in the tumor tissue of DEN-treated mice (Fig. 2). This increase in 4N cells affected only the tumor tissue and not the normal surrounding tissues or the livers of untreated Ahr−/− mice. These data indicate that basal hepatocyte ploidy control is unaffected by loss of Ahr in the normal regions of livers from mice exposed to DEN, but that DEN exposure significantly impairs ploidy distribution in the preneoplastic tissue of AHR-deficient livers.

**Loss of Ahr promotes increases in IL-6 and TNFα expression.** Inflammatory cells and proinflammatory cytokines found in tumors are major contributing factors to a state of chronic inflammation more likely to promote tumor growth, progression, and immunosuppression than they are to mount an effective host antitumor response (37, 38). Serum levels of IL-6, a multifunctional cytokine largely responsible for the hepatic "acute phase response," and TNFα, a major mediator of inflammation, are highly elevated in experimental models of HCC in mice (32, 39) and in a number of human conditions believed to be HCC precursors (40). To determine whether the Ahr genotype plays a significant role in the increase of inflammatory cytokine expression observed by others in DEN-treated mice (32), we used real-time RT-PCR and ELISA to measure the levels of IL-6 and TNFα mRNAs and proteins as a function of genotype and treatment to the male mice. Expression of both cytokines was already significantly higher in Ahr−/− mice than in their Ahr+/+ counterparts, even in animals that had not been treated with DEN. Both IL-6 and TNFα mRNAs were significantly increased in mice of both genotypes already as early as 3 weeks after DEN injection (Fig. 3A), reaching nearly 5-fold increases over untreated controls by 36 weeks after treatment (Fig. 3B). Parallel determinations of the corresponding proteins in the same tissues showed a similar pattern of significantly higher levels of IL-6 and TNFα in liver lysates of Ahr−/− at both 3 and 36 weeks and induction in livers of both genotypes after DEN treatment (Fig. 3C and D). These data suggest that AHR deficiency may promote a proinflammatory state by upregulating the expression of these two cytokines and that this condition is exacerbated by DEN treatment.

**Loss of Ahr increases expression of proliferative markers and represses tumor suppressor genes.** Direct comparison of the transcriptional characteristics of mouse and human HCC has allowed the identification of a limited number of genes that accurately predict the length of survival and provide new molecular insights into the pathogenesis of HCC (41). Comparative global gene expression profiling has provided important insights in the identification of genes associated with clinicopathologic features of HCC, refining the diagnosis and prognostic predictions of HCC patients (41). Of particular interest in this regard is the expression of cell proliferation genes, which in DEN-induced mouse HCC is most similar to their expression in the poorer prognosis group of human HCC (42). To investigate whether AHR plays a significant role in the aberrant gene expression patterns associated with DEN-induced tumorigenesis, we examined the expression of survival and proliferation genes in hepatocytes from control tissues, DEN-induced tumors, and normal tissues adjacent to the tumors. We found that most tumor suppressor genes and genes such as Cdc25b and Cdc25c, involved in the activation of cyclin-dependent kinases, were expressed at similar levels in control tissues of both mouse strains. In contrast, whereas the Cdc25 genes were upregulated by DEN treatment, the tumor suppressor genes were repressed in normal and tumor hepatocytes of Ahr−/− mice to a larger extent than in Ahr+/− mice. Among the proapoptotic genes, Apaf1 was upregulated in control and normal tissues of DEN-exposed mice but was downregulated in the tumors; p73 and TA-p73, its proapoptotic variant, were upregulated
in control Ahr−/− hepatocytes but were repressed in normal and tumor tissues of DEN-treated Ahr−/− relative to wild-type mice. Sirt1, E2f1, and Rb were repressed in all three conditions tested, indicating that Ahr ablation is responsible for their downregulation. On the other hand, Tp53 is upregulated in hepatocytes of control mice and is repressed as consequence of DEN treatment in Ahr−/− mice. The three Tgfb genes seem to be already upregulated in control Ahr−/− hepatocytes; in DEN-treated mice, Tgfβ2, but not Tgfβ1 or Tgfβ3, is further induced in the tumor tissues of Ahr−/− mice. Gadd45a, a gene inducible by DNA damage, was highly expressed in control Ahr−/− hepatocytes, and its expression nearly doubled in normal and tumor tissues of DEN-treated Ahr−/− relative to Ahr+/+ mice (Fig. 4). With few exceptions, these data are consistent with the conclusion that the lack of AHR results in a type of tumor that more closely resembles the poor-prognosis phenotype of human HCC, suggesting that AHR may provide the protection characteristic of a tumor suppressor gene.

Discussion

The results presented in this study show that ablation of the Ahr gene leads to a significant increase in DEN-dependent liver tumor incidence in male mice relative to wild-type Ahr+/+ littermates of the same gender. There was no comparable change observed in female mice, in agreement with work in the literature (32). The increase in males results from higher incidence, higher nodule multiplicity, and larger nodule size. The loss of the Ahr gene seems to sensitize liver hepatocytes to acquire a phenotype that is both pro-proliferative and antiapoptotic when exposed to a...
DEN challenge, possibly due to a significantly higher constitutive level of DNA damage, as shown by γH2AX immunostaining. Our previous cell culture work has shown that lack of AHR in fibroblasts creates a condition of heightened cellular oxidative stress that triggers significant increases in DNA damage (13). We surmise that a similar condition pertains to the hepatocytes of Ahr−/− mice, and that the higher prooxidant status of these mice becomes exacerbated by DEN, itself a DNA-damaging alkylating agent. In support of this contention is the observation that expression of the Gadd45a gene, a stress sensor inducible by DNA damage, is already high in control Ahr−/− hepatocytes and in tumor-adjacent tissues of the same mice, and becomes even higher in the tumor tissue themselves.

Exposure of cells in culture to genotoxic agents results in the development of abnormal ploidy (35, 36). Although, in specific cases, aneuploidy may act as a suppressor of tumorigenesis (43), genomic instability is more likely to drive, rather than suppress, cancer (34, 43). It is likely that the sustained level of DNA damage found in Ahr-deficient hepatocytes is responsible for the abnormally high level of tetraploid hepatocytes in these mice and the ensuing increase in liver tumors. There is now compelling evidence showing that tumors often contain a near tetraploid number of chromosomes, and that the uncontrolled proliferation of tetraploid cells can trigger tumor formation (44).

Expression of the proinflammatory cytokines IL-6 and TNFα was also higher in untreated AHR-deficient than in AHR-proficient mice. The difference was maintained at the same level over the whole experimental period of 35 weeks and was greatly increased by DEN treatment. Sustained oxidative stress and DNA damage are likely to contribute to a general state of cell damage and destruction, generating tissue necrosis and inducing the expression of proinflammatory cytokines. Greater expression of these cytokines in Ahr−/− hepatocytes might be a response involved in cell survival.

DEN may aggravate this preexisting cellular condition by creating a more extensive and long-lasting state of DNA damage. However, by 35 weeks after DEN treatment, mice of both genotypes show similar levels of these two cytokines, suggesting that the difference in tumorigenesis between the two genotypes may be independent of the damage caused by sustained endogenous cytokine expression.

Expression of typical cell proliferation markers, such as the cyclin-dependent kinase inhibitors and Rb, E2f1, and Tp53, is repressed in liver tumors and tumor-adjacent tissues of DEN-treated Ahr−/− mice. In the case of Rb and E2f1, repression is also evident in hepatocytes from control mice, suggesting that AHR modulates Rb and E2f1 expression and that Ahr ablation is sufficient to downregulate these two genes, whereas DEN exposure further represses Rb expression. In contrast, the genes coding for cyclin B1 and the Cdc25 phosphatases are upregulated in those mice. These results are consistent with the classification of DEN-induced HCC in mice as having a biological phenotype similar to the poor-prognosis subclass of human HCC, characterized by the upregulation of proliferation markers (41). As in that human subclass, proapoptotic markers are downregulated in the tumors of Ahr−/− mice. The Tgfβ genes are also overexpressed in tumors and normal tissues of DEN-treated Ahr−/− mice and in untreated controls relative to the corresponding Ahr+/+ mice. This may be a unique feature of HCC in AHR-deficient mice, setting them aside from the human HCC-like class, being the result of the overexpression of Tgfβ genes in Ahr−/− mice (15). TGFβ overexpression may be at the root of the greater tumorigenesis of the Ahr−/− genotype. It seems that TGFβ has a dual role during tumorigenesis, functioning as a tumor suppressor in earlier tumor phases and turning into a tumor promoter in later phases of tumorigenesis (45). The switch occurs as a consequence of its being produced in high amounts in the tumor, which stimulates tumorigenesis by allowing tumor cells to escape immune
surveillance (46) and to promote angiogenesis (47). In agreement with the regulatory role of TGF\(\beta\) in Pit1 transcription (48), we find a concomitant increase in Pit1 expression. Pit1 encodes plasminogen activator inhibitor serpin A1, whose expression increases migration and epithelial-mesenchymal transition and is highly elevated in many invasive tumors, including breast, brain, and gastric cancers (49, 50).

In summary, our studies show that AHR functions as a tumor suppressor that modulates the tumorigenic effect of DEN and causes a lower incidence of tumors than that found in mice lacking this protein. This AHR function is radically different from the pro-oncogenic role that AHR plays as an activator of the carcinogenic potential of its procarcinogenic ligands. Because, as discussed earlier, DEN is not an AHR ligand or a substrate for AHR-regulated cytochrome P450s, this effect is not the result of AHR-mediated DEN detoxification. Rather, it is more likely that the combination of a higher level of oxidative stress and sustained TGF\(\beta\) expression—two molecular events that may be causally connected— is responsible for the heightened tumorigenicity of the \(Ahr^{-/-}\) genotype. High constitutive levels of oxidative stress and TGF\(\beta\) expression might be a further phenotypic characteristic to be taken into consideration when assessing the prognosis of humans with HCC. This phenotype need not be solely associated with AHR deficiency but may also result from activation of different oncogenic pathways.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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