Regulation of expression of drug-metabolizing enzymes by oncogenic signaling pathways in liver tumors: a review

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Received 21 March 2019; received in revised form 23 May 2019; accepted 24 June 2019

KEY WORDS
Xenobiotic metabolism; Hepatocytes; WNT/β-catenin signaling; RAS/MAPK signaling; Gene mutation; Cytochrome P450

Abstract
Mutations in genes encoding key players in oncogenic signaling pathways trigger specific downstream gene expression profiles in the respective tumor cell populations. While regulation of genes related to cell growth, survival, and death has been extensively studied, much less is known on the regulation of drug-metabolizing enzymes (DMEs) by oncogenic signaling. Here, a comprehensive review of the available literature is presented summarizing the impact of the most relevant genetic alterations in human and rodent liver tumors on the expression of DMEs with a focus on phases I and II of xenobiotic metabolism. Comparably few data are available with respect to DME regulation by p53-dependent signaling, telomerase expression or altered chromatin remodeling. By contrast, DME regulation by constitutive activation of oncogenic signaling via the RAS/RAF/mitogen-activated protein kinase (MAPK) cascade or via the canonical WNT/β-catenin signaling pathway has been analyzed in greater depth, demonstrating mostly positive-regulatory effects of WNT/β-catenin signaling and negative-regulatory effects of MAPK signaling. Mechanistic studies have revealed molecular interactions between oncogenic signaling and nuclear xeno-sensing receptors which underlie the observed alterations in DME expression in liver tumors. Observations of altered DME expression and inducibility in liver tumors with a specific gene expression profile may impact pharmacological treatment options.

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1. Introduction

The group of drug-metabolizing enzymes (DMEs) consists of families of enzymes which are involved in the metabolic conversion of both endogenous and exogenous compounds. The latter are often referred to as “xenobiotics” and may comprise drugs, pesticides, herbicides, food additives, and many environmental chemicals of all kind. According to their primary function the underlying proteins are grouped into metabolic enzymes involved in the so-called phase I (target functionalization) and phase II (target conjugation with endogenous molecules) of xenobiotic metabolism, while functionally related transporters of phase 0 and phase III are responsible for the uptake of xenobiotics into cells, or for the active excretion of metabolites out of cells, respectively. For an overview of xenobiotic metabolism see Fig. 1. In mammals, the level and activity of DMEs is highest in the liver; however, many DMEs are also expressed in other organ systems such as for example the gastrointestinal tract. This review will concentrate on the liver and on enzymes of phases I and II.

A key group of proteins involved in phase I of xenobiotic metabolism are enzymes belonging to one of the various cytochrome P450 (CYP) subfamilies. In the early 70s of the last century, it was discovered that the content of some CYPs was decreased in experimentally induced hepatomas in rats as compared to normal liver. Present day omics analysis on global gene expression patterns demonstrates a similar decrease in CYP expression in human hepatocellular carcinoma (HCC); a comprehensive meta-analysis is available by use of the HCCDB database which is available at: www.lifeome.net/database/hccdb/home.html. Based on the observation of decreased DME-expression in liver tumors, it was postulated that preneoplastic and neoplastic cells are less sensitive to the toxic action of 2-acetylaminofluorene and other hepatocarcinogens which need metabolic activation of the parental compound to toxic intermediates by CYP enzymes. Based on this observation Farber’s group developed the so-called Solt-Farber model which allows for rapid induction of neoplastic nodules in rat liver based on selective pressure given by 2-acetylaminofluorene on preneoplastically transformed hepatocytes produced by single injection of a strong hepatocarcinogen such as N-nitrosodiethylamine. While potentially toxin-activating enzymes such as CYPs are generally decreased in hyperplastic nodules and hepatomas, preferentially detoxifying enzymes such as microsomal epoxide hydrolase (mEH), glucose-6-phosphate-dehydrogenase (G6PD), and UDP-glucuronosyltransferases (UGTs) were found to be increased in premalignant lesions. This further confers a selective advantage to the preneoplastic and neoplastic liver cells and led to the “selective toxicity resistance” model postulated by Farber to be a generalized model for chemical hepatocarcinogenesis.

In a comprehensive immunohistochemical study, Buchmann et al. demonstrated that the decreases in phase I enzymes (shown for two phenobarbital (PB)-inducible and two 3-methylcholanthrene-inducible CYPs) along with increases in phase II enzymes (including cytosolic GSTs B and C, and mEH) occurred early during the carcinogenic process in rat liver, presumably already during its initiation. Since individual lesions showed heterogeneity in DME expression and since some of the DMEs in the preneoplastic lesions were still inducible by PB, it was suggested that the focal enzyme alterations result from genotoxic effects of the carcinogen on “regulatory systems of a higher order” rather than from mutational events in individual genes encoding DMEs. The nature of these higher order regulatory systems operative in the rat liver lesions was entirely unknown at the time and remained obscure during the following decades. Then, activating mutations in Ctnnb1, encoding β-catenin, were

![Figure 1](image)

**Figure 1** Overview of the phases and important enzymes and transporters of xenobiotic metabolism in hepatocytes. Functionalization in phase I is followed by conjugation to endogenous substrates in phase II and excretion in phase III of the biotransformation process. Abbreviations: COX, cyclooxygenase; CYP, cytochrome P450; EH, epoxide hydrolase; GST, glutathione-S-transferase; MAO, monoamine oxidase; MDR, multi-drug resistance protein; MRP, multi-drug resistance-related protein; NAT, N-acetyltransferase; NQO, NAD(P)H-quinone oxidoreductase; OAT, organic anion transporter; OATP, organic anion-transporting peptide; OCT, organic cation transporter; UGT, UDP-glucuronosyltransferase.
found to be present in about 30% of chemically induced rat liver
tumors\textsuperscript{11} which corresponds in frequency to CTNNB1 mutations
found in HCC\textsuperscript{12}. Mutation of Cnnb1 is associated with consti-
tutive activation of the canonical WNT/\(\beta\)-catenin signaling
pathway which, for reasons discussed later, is very unlikely to be
responsible for down-regulation of CYP enzymes observed in the
rat liver tumors. Mutations in one of the Ras oncogenes, frequently
detected in mouse liver tumors, would in principle be
much better candidates for reduction of CYP enzymes, but are
very rarely present in rat and human liver tumors.

2. Species differences in mutational patterns of liver tumors

The genes most frequently affected by mutation in human\textsuperscript{12},
rat\textsuperscript{11,13,14} and mouse\textsuperscript{15,16} primary liver tumors do show some
overlap but also divergence, as summarized in Table 1. The rea-
sons for the observed species-specific differences in the muta-
tional patterns of the driver genes affected are not known.
However, part of it may be linked to differences in the etiology of
the tumors: while rodent liver tumors were mostly experimentally
induced by the use of known hepatocarcinogenic chemicals, the
occurrence of liver tumors in humans is mostly linked to chronic
hepatitis B and C virus infection, alcohol abuse and, to a minor
part, to exposure to aflatoxins. In addition, species-specific dif-
fferences in the biology underlying tumor manifestation and pro-
gression are likely to play a role. While primary liver tumors in
humans reflect a very heterogeneous group of neoplasms with
distinctive clinical and pathologic features\textsuperscript{17}, liver tumors in mice
are much more homogeneous in appearance. In the following, we
will very briefly discuss effects produced by the mutational
changes on cellular signaling pathways before we discuss their
consequences for DME expression and drug metabolism in the
affected tumor cells.

2.1. Human liver tumors

TERT (telomerase reverse-transcriptase, coding for the catalytic
subunit of telomerase) promoter mutations are the most frequent
genetic alterations found in human primary liver tumors and one
of the earliest genomic events in human liver carcinogenesis\textsuperscript{12,18}. TERT
promoter mutations are a common feature of human cancers and
are predicted to increase promoter activity and TERT transcrip-
tion. In fact, in contrast to normal liver, TERT activity is
restored in over 90% of human HCCs investigated\textsuperscript{15}. Interestingly,
TERT promoter mutations are often found together with mutations
in a second gene frequently mutated in human HCC, namely
CTNNB1\textsuperscript{19,20}, which encodes the oncoprotein \(\beta\)-catenin, a mem-
ber of the WNT signaling pathway (see below). The available data
suggest that TERT promoter mutations and activation of the WNT/
\(\beta\)-catenin pathway cooperate in HCC progression in humans\textsuperscript{18}. Underlying
cause may be a recently discovered cross-talk between TERT and the WNT/\(\beta\)-catenin pathway, in which telomerase functions in a “non-canonical” fashion as a cofactor in the \(\beta\)-
catenin transcriptional complex, as reviewed by Li and Tergaon-
kar\textsuperscript{21}, resulting in activation of WNT/\(\beta\)-catenin-dependent
transcription. Interestingly, this cofactor-function of TERT is
mediated by BRG1, a protein of the SWI/SNF (SWItch/Sucrose
Non-Fermentable) complex required for chromatin remodeling.
Other SWI/SNF members include ARID1A and ARID2, as dis-
cussed below.

TP53 encodes the tumor suppressor protein p53, which has a key
function in controlling, amongst others, the induction of
senescence and apoptosis; for review see e.g. Hafner et al.\textsuperscript{22} or
Mello and Attardi\textsuperscript{23}. P53 is known to mediate cellular senescence,
following e.g. the inappropriate activation of oncogenic signaling
pathways, which explains why TP53 is frequently inactivated by
mutation in HCC and many other human cancers. Among the
various oncogenic pathways that may trigger a p53-senescence-
inducing response is the WNT/\(\beta\)-catenin pathway, constitutively
activated by mutation of CTNNB1\textsuperscript{24}.

CTNNB1 and its rodent ortholog Cnnb1 encode \(\beta\)-catenin, a central player in the canonical WNT/\(\beta\)-catenin signaling pathway.
Cytosolic levels of \(\beta\)-catenin are stringently regulated by a multi-
protein complex, which mediates phosphorylation of the protein
thus initiating its ubiquitinylation and subsequent degradation by
the proteasome; for review see Lustig and Behrens\textsuperscript{25}. Mutation of
one of the phosphorylation sites leads to \(\beta\)-catenin accumulation
followed by nuclear transfer, where it associates with transcription
factors of the T-cell factor (TCF)/lymphoid enhancer factor family
and induces target gene transcription. Part of the cytosolic \(\beta\)-
catenin degradation complex is AXIN1, the gene of which is also
mutated in a certain fraction of human HCC\textsuperscript{12}.

ARID (AT-rich interactive domain-containing protein) 1A and 2 are
both members of the ATP-dependent chromatin remodeling
SWI/SNF complex, which is required for transcriptional activation
of genes normally repressed by chromatin; for review see Savas
and Skardasi\textsuperscript{26}. ARID1A (also termed BAF250a) and ARID2 are
frequently mutated in diverse human cancers including HCC\textsuperscript{12}.
Even though ARID1A and 2 are generally assumed to act as tumor
suppressors, their role in HCC development is not entirely clear,
since up-regulation of ARID1A expression is observed in a
considerable number of HCC\textsuperscript{27}. Deficiency in Arid1a in the
respective knockout mouse induces steatohepatitis and HCC\textsuperscript{28}.
However, since ARID1A was highly expressed in the primary
tumors but was lost in expression in metastatic HCC cases, it may
promote carcinogenesis during the early phases of tumor initiation
but suppress tumor progression in late-stage HCC\textsuperscript{29}.

It is interesting to note that mutations in one of the oncogenic
Ras genes, which are very frequently mutated in mouse liver tu-
mors\textsuperscript{15,30}, are only very rarely observed in human and rat liver
tumors\textsuperscript{31}. The reason for this species difference is not known, but
despite the lack of Ras mutations, activation of RAS-downstream
mitogen-activated protein kinase (MAPK) signaling is observed in
50%–100% of human HCC and is associated with poor
prognosis\textsuperscript{32}.

Another interesting note is that many of the genes recurrently
mutated in human HCC encode proteins that have a direct or
indirect link to \(\beta\)-catenin, which plays a central role in regulation of
DME expression in hepatocytes, as will be discussed in detail later.

Table 1. Genes frequently affected by mutation in human
and rodent primary liver tumors.

| Human | Rat        | Mouse     |
|-------|------------|-----------|
| TERT  | Nrf2/Keap1 | Hras      |
| TP53  | Ctnb1      | Braf      |
| CTNNB1/AXIN1 | Tp53 | Ctnb1 \textsuperscript{19} |
| ARID1A/2 | Egfr       |           |

\textsuperscript{*Ctnb1 mutations specifically found after tumor promotion with
PB-like compounds.}
2.2. Rat liver tumors

NRF2/KEAP1: Following the observation that in about 6%–8% of cases human HCCs harbor mutations in either the NFE2L2 gene encoding NRF2 [Nuclear factor (erythroid-derived 2)-like 2], or in KEAP1 which encodes the NRF2 inhibitor KEAP1 (Kelch-like ECH-associated protein 1), respectively [33,34], rat liver tumors were also screened for mutations in the two underlying genes [13]. In this study, pre-neoplastic lesions as well as tumors of differing stages were induced by a modified Solt-Farber protocol including 2-acetylaminofluorene as selective chemical. More than 70% of preneoplastic lesions and about 60%–80% of HCCs were found to harbor mutations in Nfe2l2 or, to a lesser extent, in Keap1 [13]. The mutations detected in Nfe2l2 or Keap1 impaired the binding between the two proteins and therefore attenuated the inhibitory activity of KEAP1 onto NRF2-mediated signaling. Therefore they should be considered as activating mutations. However, whether NRF2 plays a pro- or anti-tumorigenic role during the early phases of the malignant process is unclear [35].

Ctnnb1 mutations are detected in about 20%–30% of chemically induced rat liver tumors [11,13]. Since no such mutations were detected in preneoplastic lesions or early HCC, Ctnnb1 mutations are likely associated with a late stage in malignant progression in rat hepatocarcinogenesis [13].

Tp53 mutations leading to inactivation of the protein as transcription factor have been described to occur in about 30%–40% of chemically induced rat liver tumors, both in pre-cancerous and cancerous lesions [14,36].

2.3. Mouse liver tumors

Hras mutations leading to the constitutive activation of the HA-RAS/p21 oncoprotein are very frequent in both, spontaneous and chemically-induced mouse liver tumors [15,37,38]. HA-RAS is a monomeric G protein which forwards mitogenic signals received by growth factor receptors to a cascade of downstream kinases, as reviewed by Sun and coworkers [39]. Interestingly, the frequency of occurrence is dependent on the susceptibility of spontaneous liver tumor development and susceptibility towards chemical induction of liver tumors which differs considerably between different mouse strains: susceptible strains show a high prevalence of Hras mutations in their liver tumors while the prevalence of such mutations is much lower in resistant strains [38].

Braf encodes a signaling protein directly downstream of HA-RAS. While Hras is predominantly mutated in mouse liver tumors from susceptible strains, the prevalence of Braf mutations is higher in resistant strains such as C57BL40. Together, mutations in either Hras or Braf are observed in more than 70% of mouse liver tumors [40]. Very likely, they occur already during initiation of the carcinogenic process [41].

Ctnnb1 mutations are the most prominent type of genetic lesion in mouse liver tumors, occurring in more than 80% of cases. However, this only applies to tumors induced by a regimen including PB or a PB-like agent as tumor promoter [16,42]; see also Fig. 2. By contrast, mouse liver tumors chemically induced under a protocol without PB-mediated tumor promotion are often Hras- or Braf-, but not Ctnnb1-mutated [16,30]. This finding strongly suggests that in mouse liver PB or similarly acting agents select for hepatocytes mutated in Ctnnb1.

Figure 2: DME characteristics of chemically induced mouse liver tumors with activating mutations in the Ctnnb1, Hras, or Braf proto-oncogenes. Spontaneous tumors or tumors induced by application of the genotoxic tumor initiator N-nitrosodiethylamine (DEN) mostly leads to tumors with activated MAPK signaling due to mutations in Hras or Braf. By contrast, chronic treatment with the tumor promoter phenobarbital (PB) or similarly acting compounds leads to the outgrowth of liver tumors with activated β-catenin due to activating Ctnnb1 mutations. Tumors with Hras and Braf mutations generally express low basal levels of DMEs (esp. CYPs and GSTs). In contrast to Hras-mutated tumors which are refractory to DME induction by constitutive androstanete (CAR) agonists, mouse liver tumors with Braf mutations respond to CAR activation with CYP and GST induction. Hepatomas with activated β-catenin display high constitutive expression of DMEs. For more details, please refer to the main text.
Interestingly, mutations in TP53/Tp53, which are very frequent in both human and rat primary liver tumors, are very rare in mouse liver tumors but occur frequently in cell lines established from the mouse liver tumors\(^5\). This evidence suggests that mutational inactivation of the murine p53 tumor suppressor does not confer a selective advantage to the mutated tumor cells. The same seems to be true for the human ortholog TP53 which, when introduced as transgene into mouse hepatocytes in vivo, is also not found inactivated by mutation in liver tumors experimentally induced in the transgenic mice\(^6\).

3. Observations on DME expression in liver tumors with specific mutational patterns

3.1. Telomerase-activation and DME expression

To the best of our knowledge, no studies about the regulation of DME expression or activity in liver tumor cells by the promoter-mutated TERT protein have been published so far. Immortalization of human fetal hepatocytes by over-expression of telomerase resulted in some changes to CYP expression, with diminished levels of CYP1A1, CYP2C9, CYP2E1, and CYP3A4, but elevated levels of CYP2B4\(^7\). In addition, indirect effects through modulation of β-catenin-dependent gene expression programs appears theoretically possible, since TERT affects transcription of WNT/β-catenin target genes through BRG1-mediated interaction with β-catenin/TCF at WNT-responsive gene promoters (for review see Li and Tergaonkar\(^21\)). Effects of β-catenin on DME expression are discussed below.

3.2. Inactivation of p53 tumor suppressor function and DME expression

To the best of our knowledge, no systematic comparisons of DME expression in TP53/Tp53 wildtype and mutant human or rodent liver tumors are available. However, in vitro evidence suggests a possible role of the p53 tumor suppressor protein in the regulation of some DMEs: a study with human liver tumor cells revealed an induction of various CYPs from families 1–3, including the important isoform CYP3A4, by p53\(^8\). Similarly, loss of p53 in mice resulted in decreased metabolism of a CYP3A substrate\(^9\). Mechanistically it appears plausible that mutations of p53 and DMEs are mediated by the transcription factor activity of p53, as has been shown for example for the human CYP2A6 promoter in human liver tumor cells in vitro\(^10\). Additional evidence suggests interactions with signaling via nuclear receptors regulating DMEs: inhibition of pregnane-X-receptor (PXR), the prototype CYP3A4-inducing nuclear receptor, by p53 has been reported\(^11\). This finding appears to contrast the above observations of p53-dependently increased CYP3A4 expression. An inhibition of the AHR and its target gene CYP1A1 by p53 has also been described, even though not in liver cells\(^12\). Thus, more research is needed to clarify the interplay of p53, nuclear receptors and DMEs under varying conditions in different cell types.

3.3. Alterations in chromatin remodeling and DME expression

ARID1A, a member of the SWI/SNF chromatin remodeling complex is often overexpressed in early HCC, while being downregulated in metastatic cancer. In mice, overexpression of ARID1A has been demonstrated to be associated with increased expression of several CYP isoforms including Cyp2e1\(^29\). Potentially, this increase in CYP expression in the tumor cells may promote the generation of reactive oxygen species mediating liver injury and hepatocarcinogenesis\(^29\).

3.4. NRF2-mediated changes in DME expression

NRF2 and KEAP1 are central within a redox-sensitive signaling system that regulates up to 10% of human genes\(^32\). Well-known target genes include those encoding reactive oxygen- or electrophiles-inactivating enzymes such as NQO1 [NAD(P) H:quinone oxidoreductase 1], heme oxygenase-1, GSTs, UGTs, and multidrug resistance-associated proteins\(^33\). Keap1 knockout mice showed an increase in NRF2 protein in liver and increases in the expression of NQO1 and GSTs\(^34\). Interestingly, there exists an intimate cross-interaction between NRF2- and aryl hydrocarbon receptor (AHR)-dependent signaling pathways (for review see Kohle and Bock\(^35\): murine Nfe2l2 is a target gene of the AHR\(^36\), while Ahr, on the other hand, is a transcriptional target of NRF2\(^37\). Therefore, the expression of Ahr and some of its downstream targets, such as Cyp1a1, Cyp1b1 and Gsta1 are higher in expression in Keap1 knockout cells\(^37\).

The level of expression of CYPs was not determined in Nfe2l2/Keap1-mutated rat liver tumors, but the NRF2 target genes Nqo1 and Gsta4 were evaluated and found to be increased\(^31\). Therefore constitutive activation of NRF2 signaling in Nfe2l2/Keap1-mutated rat liver tumors might potentially explain the observed upregulation of (some) phase II enzymes including GSTs and UGTs. It does not explain, however, why phase I enzymes including CYPs are reduced in expression in these tumors. Rather, one would expect an increase in expression of e.g. CYP1A isoforms, which was not observed in any of the studies where CYP expression was analyzed. Therefore, other “higher order regulators” must play a role in the regulation of these enzymes in rat liver tumors.

3.5. Activation of the WNT/β-catenin signaling pathway and associated changes in DME expression

In 2005 our group was, to the best of our knowledge, the first to report on the positive regulatory activity of WNT/β-catenin signaling on the expression of CYP enzymes in liver cells\(^38\), see also Fig. 2. This conclusion was based on the observation that mouse liver tumors harboring activating mutations in the Ctnnb1 gene, encoding β-catenin, showed higher levels of several CYP isoforms (CYP1A1, CYP2B, CYP2C and CYP2E1 proteins), while Ctnnb1 wildtype tumors exhibited decreased levels of these CYP isoforms\(^39\). The increase in CYP protein level corresponded to increases in the respective mRNAs indicating that mutation of Ctnnb1 leads to transcriptional activation of a number of CYP isoforms in mouse liver tumors. In the initial studies, the Ctnnb1-mutated tumors in mouse liver were generated by a sequential initiation-promotion regimen: for tumor initiation, mice were first treated with a single dose of N-nitrosodiethylamine, which is converted in hepatocytes to an electrophilic DNA-reactive mutagen; this was then followed by chronic treatment with PB acting as a tumor promoter. PB, however, is also a potent DME inducer. Increased expression of CYPs and other DMEs could therefore, in principle, also result from inducing effects of PB in the Ctnnb1-mutated mouse liver tumors. Later studies, however, confirmed that Ctnnb1-mutated mouse liver tumors show
increased DME expression, even after PB had been withdrawn, demonstrating that activated β-catenin by itself is sufficient to drive DME expression in mouse liver (unpublished observation).

This was substantiated in a transgenic mouse strain with hepatocyte-specific expression of a point-mutated, constitutively active version of β-catenin, where strongly elevated CYP levels were seen even in periporal hepatocytes, where these enzymes normally are not expressed69,70.

In wildtype liver, CYPs and other important DMEs are preferentially expressed in perivenous hepatocytes67 which also display physiological activation of the WNT/β-catenin pathway61,62. It was demonstrated that the preferential perivenous expression of DMEs in mouse liver is regulated by WNT/β-catenin-activating signals derived from the endothelial cells of the central veins65,66. In line with the aforementioned observations, results from studies conducted by several different groups including ours demonstrated that various CYP isoforms, especially CYP2E1 and CYP1A, are no longer expressed at the mRNA and protein level in livers of mice with conditional hepatocyte-specific knockout of Ctnnb165,66. Similarly, down-regulation of a number of GSTs from phase II of xenobiotic metabolism67 and of enzymes engaged in the synthesis of the CYP prosthetic group heme68 were observed in that mouse model. Experiments with xenobiotic inducers of DMEs demonstrated that the knockout of Ctnnb1 resulted in diminished DME induction following exposure to xenobiotics acting via activation of the receptors CAR or AHR66,69. These results clearly demonstrate that WNT/β-catenin signaling is a key player in regulating CYP expression in Ctnnb1-mutated mouse hepatocytes.

Less is known about human liver tumors. In human hepatoblastoma, a pediatric tumor very frequently mutated in CTNNB1, the human ortholog of mouse Ctnnb1, up-regulation of various CYP isoforms (in particular CYP2E1) was observed in the epithelial parts of the tumors70. By contrast, most CYPs are generally down-regulated in human HCCs71,72. Of note, in vitro analyses with human HepaRG hepatocarcinoma cells and primary human hepatocytes demonstrated transcriptional regulation of a number of CYPs, especially CYP2E1, by WNT/β-catenin signaling73,74. In a study from our group, Ctnnb1-mutated and Ctnnb1-wildtype mouse liver tumors were analyzed in parallel with human CTNNB1-mutated or -wildtype HCCs75. Glutamine synthetase, a biomarker for increased β-catenin-mediated signaling, and various CYP isoforms were increased in expression in the Ctnnb1-mutated tumors from mice as compared to the surrounding normal liver tissue75. By contrast, while glutamine synthetase was also over-expressed in the CTNNB1-mutated human HCCs, all CYP isoforms investigated were lower in expression in the tumors when compared to normal liver (unpublished observation). However, when CYP expression levels in the CTNNB1-mutated HCCs were compared to those in the corresponding CTNNB1-wildtype tumors, higher expression levels were detected in CTNNB1-mutated tumors (unpublished observation). Interestingly, CTNNB1 mutations were only seen in HCCs associated with hepatitis C virus infection but not in those associated with hepatitis B virus75. This preference of CTNNB1 mutations in HCC with hepatitis C virus association has also been observed by others (e.g. see Pezzuto et al.76 or Tornesello et al.77). This is of interest, since other studies have reported higher expression of CYPs, in particular CYP2E1, in human hepatitis C virus—as compared to hepatitis B virus-associated HCCs7,78.

3.6. Effects on DME expression upon activation of Ras-downstream signaling

Hras mutations are frequent in spontaneous and chemically-induced mouse liver tumors, particularly in those strains showing a high background prevalence of liver tumor formation78. However, mutations in Hras or one of the other oncogenic Ras genes, Kras or Nras, are only very rarely observed in rat or human liver tumors79. Nonetheless, frequently detected activation of RAS downstream kinases in human HCCs80 argue for a relevance of activation of this signaling pathway also in human liver tumors.

With regard to DME expression, the situation in Hras-mutated mouse liver tumors is quite clear: many important enzymes from phase I and II are strongly down-regulated in expression in these tumors at the mRNA and protein levels (Fig. 2). This phenomenon is similarly observed in mouse liver tumors with mutations in Braf, which are indiscernible from their Hras-mutated cousins in terms of global transcriptomic or proteomic expression patterns5,79,80. Nonetheless, it has been observed that Hras-mutated mouse liver tumors are refractory to DME induction via activation of the constitutive androstane receptor (CAR), whereas Braf-mutated tumors responded to the presence of the CAR activator PB with pronounced induction of CYPs and GSTs75. Studies with transgenic mice expressing a mutationaly activated human HRAS oncogene in some hepatocytes show that the perivenous gene expression profile, including the expression of important DMEs, is abolished in liver cells with activated HA-RAS58,82.

4. In vitro studies and mechanistic considerations

4.1. WNT/β-catenin signaling

A number of molecular mechanisms have been identified by which signaling through the WNT/β-catenin pathway regulates the transcription of different DMEs (Fig. 3). First, the xenosensing receptors CAR and AHR are transcriptionally regulated by the β-catenin pathway68,69,83-86. This way, activated β-catenin may contribute to elevated levels of DME-regulating receptors. Nonetheless, in vitro analyses suggest that AHR up-regulation by β-catenin activation might not be crucial for the observed effects of β-catenin signaling on the expression of the model AHR target gene Cyp1a187. Second, direct transcriptional activation of DMEs by the β-catenin/TCF transcription factor complex has been demonstrated by in vitro gene promoter analyses, for example in case of murine Cyp2e1 and human CYP1A189,87,88. Cyp2e1 and Cyp1a2 promoter occupancy by β-catenin/TCF has also been confirmed in mice in vivo30,88. Third, there is in vitro evidence for a cooperative behavior of β-catenin/TCF and the AHR at the human CYP1A1 promoter, where specific binding sites for these transcription factors are located in close proximity89,87,88. Similarly, a cooperation of transcription factor binding sites for hepatocyte nuclear factor 1 alpha (HNF1α) and β-catenin/TCF has been shown for the mouse Cyp2e1 promoter89. Fourth, β-catenin enhances the transcriptional activity of the AHR at its binding sites at the DNA89. The molecular basis of the decreased response of Ctnnb1 knockout hepatocytes to CAR activators64,85,90 remains to be studied. In summary, the β-catenin pathway constitutes a master positive regulator of DME expression in hepatocytes, acting through a variety of different molecular mechanisms, especially via a complex interplay with xenobiotic-sensing nuclear receptors; for review see also Braeuning and Schwarz91 or
Moreover, there is evidence that β-catenin activation in mouse liver is able to suppress the DME-repressing signaling program orchestrated by signaling through the MAPK cascade via the induction of dual-specificity phosphatases (DUSPs), negative regulators of the RAS/MAPK pathway. This phenomenon is not observed in tumors with mutationally activated B-Raf, where ERK phosphorylation is much less pronounced. Antagonistic action of the DME-inhibiting mitogen-activated protein kinase (MAPK) pathway and the DME-inducing β-catenin pathway have been described, for example via the induction of dual-specificity phosphatases (DUSPs) by the β-catenin pathway. For more details, please refer to the main text.

4.2. RAS/RAF/MAPK-dependent signaling

Much less is known about the molecular mechanisms by which activation of the MAPK signaling pathway due to mutations in Hras or Braf is able to suppress DME expression (Fig. 3). Observations from mouse liver tumors growing directly next to a branch of the hepatic central vein show that the perivenous gene expression program, including DME expression, which is normally activated in perivenous hepatocytes in close contact to the venous endothelial cells, is not getting activated in Hras-mutated tumor cells. This indicates that MAPK-dependent signaling has the ability to block the activation of the β-catenin pathway. Similarly, perivenous-specific gene expression is abolished in hepatocytes from a transgenic mouse model expressing constitutively active HRAS in a fraction of perivenous hepatocytes. This indicates that MAPK signaling is able to suppress activation of the DME expression-promoting β-catenin signaling pathway. Based on the antagonistic behavior of both signaling pathways, it has been proposed that gradients of MAPK- and β-catenin-dependent signaling regulate DME expression along the porto-central axis in healthy liver. Down-regulation of DMEs in liver is also observed under conditions of chronic inflammation. In this case, suppression of DME expression is mediated by release of inflammatory cytokines which act on receptors located e.g. upstream of the RAS/MAPK pathway. Overexpression of the oncoprotein C-MYC, often seen in human HCC, may also be part of cytokine-mediated DME-repression in liver, and may also explain down-regulation of DME expression in HCC. Furthermore, interference of interleukins with drug-metabolizing enzymes has been observed, which is based on an inhibition of the retinoid X receptor, the dimerization partner of several nuclear receptors involved in DME regulation.

A very interesting observation is the fact that Hras- and Braf-mutated mouse liver tumors, even though highly similar with respect to their basal gene expression levels, behave strikingly different when exposed to PB, a model xenobiotic inducer of CAR-dependent gene expression: transcriptional induction of various CYPs and GSTs is observed in Braf-mutated tumors, but not or only to a very limited degree in their Hras-mutated cousins. The underlying reason of the latter phenomenon is likely to be the difference in the activating phosphorylation of extracellular signal-regulated kinase (ERK) 1/2, an important kinase within the MAPK cascade: ERK phosphorylation is detected at very high levels in Hras-mutated tumors, whereas the degree of ERK phosphorylation is considerably lower in Braf-mutated tumors. It has been previously reported that CAR-mediated functions are counteracted by ERK activation, as the phosphorylated kinase retains the constitutive androstane receptor (CAR) in the cytosol to inhibit DME induction by CAR agonists. Thus, the different levels of ERK phosphorylation may explain the differences between the two tumor types when exposed to a CAR activator.

5. Conclusions

Mutations in key proto-oncogenes or tumor suppressor genes trigger specific downstream gene expression profiles in the respective tumor cell populations, with important DMEs being...
part of the gene batteries regulated by oncogenic signaling. Our synopsis of published literature demonstrates that still a lot of research is needed to fully understand the mechanisms by which oncogenic signaling pathways affect the expression of DMEs in human liver tumors. Most information is available with respect to the oncogenic WNT/β-catenin and MAPK-dependent signaling cascades, which show largely opposing effects on DME expression. Several molecular mechanisms, especially interactions with nuclear xeno-sensing receptors, have been identified by which oncogenic signaling can affect DMEs at the transcriptional level. Many anticancer drugs, including novel targeted therapeutics, are subject to metabolism by DMEs. Thus knowledge on the connection of oncogenic signaling and DME expression may provide information relevant for tumor therapy. For example, it has been demonstrated that the WNT/β-catenin-dependent up-regulation of Cyp2e1 in chemically induced mouse liver adenomas renders these tumors susceptible to selective poisoning with acetaminophen190. Future research will show to which degree oncogene-induced changes in DME expression may be utilized for the optimization of anti-neoplastic therapy.

Author contributions

Both authors, Michael Schwarz and Albert Braeuning, have jointly written the paper based on an intense literature survey conducted by both authors. Both authors have seen and approved the final article.

Conflict of interest

Authors declare absence of conflict of interest.

Appendix A  Supporting information

Supporting data to this article can be found online at https://doi.org/10.1016/j.apsb.2019.06.013.

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