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REVIEW

Distinctive pharmacological differences between liver cancer cell lines HepG2 and Hep3B

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Abstract As cellular models for in vitro liver cancer and toxicity studies, HepG2 and Hep3B are the two most frequently used liver cancer cell lines. Because of their similarities they are often treated as the same in experimental studies. However, there are many differences that have been largely over-sighted or ignored between them. In this review, we summarize the differences between HepG2 and Hep3B cell lines that can be found in the literature based on PubMed search. We particularly focus on the differential gene expression, differential drug responses (chemosensitivity, cell cycle and growth inhibition, and gene induction), signaling pathways associated with these differences, as well as the factors in governing these differences between HepG2 and Hep3B cell lines. Based on our analyses of the available data, we suggest that neither HBx nor p53 may be the crucial factor to determine the differences between HepG2 and Hep3B cell lines although HBx regulates the expression of the majority of genes that are differentially expressed between HepG2 and Hep3B. Instead, the different maturation stages in cancer development of the original specimen between HepG2 and Hep3B may be responsible for the differences between them. This review provides insight into the molecular mechanisms underlying the differences between HepG2 and Hep3B and help investigators especially the beginners in the areas of liver cancer research and drug metabolism to fully understand, and thus better use and interpret the data from these two cell lines in their studies.

Keywords Differences · HepG2 · Hep3B · Pharmacological · Determinants

Introduction

Primary liver cancer, predominantly hepatoblastoma (HB) and hepatocellular carcinoma (HCC), is one of the most common solid tumors, ranking fifth in incidence rate and third in cause of mortality worldwide (Calvisi et al. 2006). For in vitro studies of this particular cancer, HepG2 and Hep3B cell lines are frequently employed as experimental models because they are not only the most popularly available and well characterized liver cancer cell lines but also share many common characteristics, thus providing a unique platform for parallel comparisons.

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Furthermore, these two cell lines are also widely used as cellular reference models in pharmaceutical studies which aim to develop new drugs and to gain insights into drug metabolism, including knowledge about involved enzymes and the drug's inhibition or induction potential. It is important to note that the two cell lines, especially HepG2, express the majority of drug-metabolising enzymes (Knasmuller et al. 1998; Castell et al. 2006). Despite the well-known similarities, there are, however, important differences between these two cell lines. First and foremost, HepG2 and Hep3B are from different ethnic origins. They often exhibit different and even opposite outcomes in response to the same pharmacological treatment under the same experimental conditions. These differential outcomes include divergences in chemosensitivity in cytotoxicity, gene expression induction, cell cycle response and biochemical effects. These diverse differences frequently cause difficulties and even confusions for many investigators, especially the beginners who are largely overshadowed by the similarities between these two cell lines in attempts to analyze and interpret their experimental data. To highlight the differences between HepG2 and Hep3B and their underlying mechanism, we searched PubMed for all the available published reports that show differences between HepG2 and Hep3B cell lines. Based on the search results, we summarize the differences between HepG2 and Hep3B in several categories including intrinsic and drug-induced gene expressions, drug-altered cell cycle, cell growth inhibition and the signal pathways that are associated with the differential drug responses described in this review. Furthermore, we analyze the major factors that may be responsible for the differences between HepG2 and Hep3B cell lines. Covering these points, this review offers a relatively comprehensive reference of the often overlooked differences between HepG2 and Hep3B cell lines, and may be of interest to both clinical and basic scientists in liver cancer research and drug development, in particular to those beginners entering these fields.

Differences between HepG2 and Hep3B

Different originations of HepG2 and Hep3B cells

HepG2 and Hep3B were originally established by Aden et al. (1979). They were isolated from liver biopsy specimens of a 15-year-old Caucasian male from Argentina with primary HB, or an 8-year-old black male from the US with primary HCC (Aden et al. 1979; Knowles et al. 1980), respectively. Both cell lines contain distinctive rearrangements of chromosome 1, and other abnormal chromosomes. But they differ in the number of chromosomes per cell as HepG2 cells contain an average of 55 (50–56) chromosomes per cell whereas Hep3B cells, 60. In addition, HepG2 is hepatitis B virus negative and non-tumorigenic, but Hep3B is hepatitis B virus positive and tumorigenic (Knowles et al. 1980; Knasmuller et al. 1998).

Differential gene expression in HepG2 and Hep3B cells

As described previously (Knowles et al. 1980; Knasmuller et al. 1998), HepG2 and Hep3B have been extensively studied for their molecular biology and biochemistry. In recent years, substantial progress has been achieved in producing sufficient data on gene expression in HepG2 and Hep3B cell lines, by using several different assay techniques including PCR (Cheng et al. 2003), Western Blotting (Gangneux et al. 2003; Kusaba et al. 2007; Wu et al. 2010), immunoprecipitation (Bressac et al. 1990), zymography (Kim and Kim 2004), or electrophoretic mobility shift assay (EMSA) (Chiao et al. 2002). Table 1 lists all those genes that have been found to express differently in HepG2 and Hep3B cell lines. Some of these genes are essential components or intermediators of signal pathways. Therefore the disparity of expression level of these genes may be responsible for the different responses to drug treatment between HepG2 and Hep3B cells as discussed below.

Differential drug effects in HepG2 and Hep3B cells

There is a great amount of experimental evidence showing that HepG2 and Hep3B cells respond differently to the same drug treatment. Table 2 shows the drug treatments that give rise to different effects on cell cycle, in terms of the cell cycle arrest at G1 or G2 stages, and apoptosis in both HepG2 and Hep3B cells, and the dependence of the effects on p53 status in HepG2 cells. Table 3 lists the drug treatments that result in different effects of growth suppression on HepG2 and Hep3B cells. Moreover, Table 4 lists drug

Table 1 Gene expression level between HepG2 and Hep3B

Gene	HepG2	Hep3B	References	HBx association	HBx independence	References
RAS	Low	High	Bose et al. (2011)	Yes	NA	Benn and Schneider (1994), Doria et al. (1995)
Fas/Apo-1 (CD95)	+	–	Cruickshank et al. (1998)	Yes	NA	Shin et al. (1999)
BCL-XL	High	Low	Li et al. (2007)	Yes	NA	Miao et al. (2006)
MMP-9	Low	High	Chung et al. (2004)	Yes	NA	Chung et al. (2004)
BAX	High	Low	Kusaba et al. (2007)	Yes	NA	Liang et al. (2007)
Cyclin D1	High	Low	Kusaba et al. (2007)	Yes	NA	Klein et al. (2003), Park et al. (2006)
Survivin	Low	High	Kusaba et al. (2007)	Yes	NA	Ng and Lee (2011)
COX-2	–	+	Cheng et al. (2003)	Yes	NA	Cheng et al. (2004)
NF-kappaB	–	+	Chiao et al. (2002)	Yes	NA	Su and Schneider (1996)
TGF-β	Low	High	Liu et al. (2000)	Yes	NA	Murata et al. (2009)
SHH	Low	High	Huang et al. (2006), Sicklick et al. (2006)	Yes	NA	Arzumanyan et al. (2012)
TGF-β type I receptor	High	Low	Liu et al. (2000)	NA	Yes	Shih et al. (2000)
SOCS-1	+	–	Kusaba et al. (2007)	NA	Yes	Bock et al. (2008)
TIMP-1 and TIMP-3	High	Low	Kim and Kim (2004)	HBV	NA	Kim and Kim (2004)
Hp	+	–	Oliviero et al. (1987)	NA	NA	
p53	+	–	Bressac et al. (1990)	NA	NA	
ALDH3	+	–	Chang et al. (1998)	NA	NA	
Secretion performance in secretome	20.7x	1x	Slany et al. (2010)	NA	NA	
Plasminogen	High	Low	Malgaretti et al. (1990)	NA	NA	
ATX	low	High	Wu et al. (2010)	NA	NA	

+: expression detectable; –: deficient/undetectable; MMP: matrix metalloproteinase; TIMP: tissue inhibitors of metalloproteinases; TGF: transforming growth factor; ALDH3: aldehyde dehydrogenase-3; Hp: haptoglobin; SHH: Sonic Hedgehog; ATX: autotaxin; NA: no direct evidence available

treatments to which HepG2 and Hep3B cells react with different sensitivities, and Table 5 shows drug treatments that cause some of the genes to alter differently in HepG2 and Hep3B cells. In Table 4, a low value of the half maximal inhibitory concentration (IC_{50}) of a drug indicates a high sensitivity to the drug treatment and vise versa, otherwise there is only a qualitative comparison available from the literature for the sensitivity to the drug treatment (low vs. high).

Differential drug responses of signaling pathways in HepG2 and Hep3B cells

Over the decades, some relevant cellular signaling pathways in the development and maintenance of liver cancer have been deciphered. Among them, the rat sarcoma (RAS)/rat sarcoma-activated factor (RAF)/MAPK-mitogen activated protein kinase, ERK kinase (MEK)/extracellular regulated kinase (ERK) pathway (RAS/RAF/MEK/ERK), the Janus kinase (JAK)/STAT (signal tranducers and activator of transcription) pathway (JAK/STAT), and the Hedgehog (Hh) pathway during hepatocarcinogenesis are the three most well-studied pathways known to respond to drug treatments differently between HepG2 and Hep3B cells (Wiesenauer et al. 2004; Calvisi et al. 2006; Sicklick et al. 2006; Kusaba et al. 2007).

RAS/RAF/MEK/ERK pathway

The RAS/RAF/MEK/ERK pathway is one of the most significant cellular signaling cascades in the development and maintenance of liver cancer. Through a series of specific phosphorylation events, this pathway

Table 2 Effects of drug treatment on cell cycle

Effect/drug treatment	HepG2	p53-dependence	Hep3B	References
AG490	G1 arrest	NA	Apoptosis	Kusaba et al. (2007)
Specific inhibition of hedgehog signaling	No effect	NA	Apoptosis	Huang et al. (2006)
Ganoderiol F	G1 arrest	NA	Much less effect	Chang et al. (2006)
Aloe-emodin	G1 arrest	+	Apoptosis	Kuo et al. (2002)
C75 (fatty acid synthase inhibitor)	G2 arrest	–	G1 arrest	Gao et al. (2006)
% of apoptotic cell by dengue virus infection	20 %	NA	90 %	Thongtan et al. (2004)
Silibinin	G1 arrest	NA	Both G1 and G2-M arrests	Varghese et al. (2005)
Doxorubicin	G1 arrest	NA	G2/M arrest	Lee et al. (2002a), Choi et al. (2008)
Apoptosis by Asiatic acid (AA)	Yes	NA	No	Lee et al. (2002b)
Apoptosis by TRAIL	No	NA	Yes	Ganten et al. (2004)
Apoptosis by IFN-β/LIGHT	No	NA	Yes	Li et al. (2007)
Apoptotis NK cells	No	NA	Yes	Kim et al. (2004)
Apoptosis by NS-398	No	NA	Yes	Cheng et al. (2003)
Apoptosis by INF + 5-FU	No	NA	Yes	Koike et al. (2006)
Apoptosis by Parvovirus H1	Less	NA	More	Moehler et al. (2001)
Apoptosis by anti-Fas antibody	Yes	NA	No	Lamboley et al. (2000)
Apoptosis by IFN-β	No	NA	Yes	Lamboley et al. (2000)
Apoptosis by thymidine kinase	More	NA	Less	Krohne et al. (2001)
Apoptosis by TGF-β	No	NA	Yes	Caja et al. (2011)

+: p53-dependent; –: p53-independent; NA: no direct evidence available

Table 3 Effects of chemicals on cell growth inhibition

Factors	HepG2	Hep3B	References
VK2	No	Yes	Matsumoto et al. (2006)
Cpd 5	No	Yes	Nishikawa et al. (1999)
EPA	Yes	No	Chi et al. (2004)
NS-398	Yes	No	Cheng et al. (2003)
Dox + U0126	Yes	No	Choi et al. (2008)
Wellferon (unit/ml)	>1,000	1	Huber et al. (1991)

VK2: vitamin K2; Cpd 5 thioether analog of vitamin K; EPA: eicosapentaenoic acid; NS-398: COX-2 inhibitor; Wellferon: human lymphoblastoid interferon

transduces extracellular signals from ligand-bound tyrosine kinase receptors, such as the epidermal growth factor receptor (EGFR), the insulin-like growth factor receptor (IGFR), the vascular endothelial growth factor receptor (VEGFR) or the platelet-derived growth factor receptor (PDGFR) into cell, starting with the activation of RAS which in turn activates serine threonine kinases of the RAF-family member (Calvisi et al. 2006). Activated RAF phosphorylates MEK kinases, which activate ERK. Once activated, ERK enters nucleus to act as a regulator of gene expression of various proteins for life processes, such as those for cell cycle progression, apoptosis, extracellular matrix remodeling, cellular motility angiogenesis and drug resistance (Wiesenauer et al. 2004). As shown in Table 1, the content of RAS, a major component of the pathway is lower in HepG2 than that in Hep3B, thus inhibitors on this pathway may induce correspondingly different outcomes in these two cell lines (Bose et al. 2011). Indeed, Wiesenauer et al. have shown that the inhibition of ERK phosphorylation and the growth suppression by ERK specific inhibitors PD098059 and U0126 are more profound in HepG2 than in Hep3B (Wiesenauer et al. 2004; Choi et al. 2008). However, the decrease in ERK protein expression by antisense knockdown is more sensitive and the

Table 4 Sensitivity of cells to drug treatment

Factors/effect	HepG2	Hep3B	References
IC_{50} of U0126 (μM)	0.5 (high)	5 (low)	Wiesenauer et al. (2004)
IC_{50} of Ar-turmerone (μg/mL)	64.8	122.2	Cheng et al. (2012)
IC_{50} of doxorubicin (μM)	0.25	0.12	Lee et al. (2002a), Choi et al. (2008)
Luteolin (μg/mL)	5	1	Chang et al. (2005)
10058-F4	High	Low	Lin et al. (2007)
Dietary genotoxins	High	Low	Majer et al. (2004)
Induction of micronuclei by AalphaC	Yes	No	Majer et al. (2004)
Styrylpyrone	High	Low	Lan et al. (2005)
KX2-391	Low	High	Lau et al. (2009)
Dasatinib	Low	High	Lau et al. (2009)
Cisplatin	High	Low	Qin and Ng (2002)
RA	High	Low	Jung et al. (2005)
ATRA	Low	High	Arce et al. (2005)

U0126: ERK inhibitor; 10058-F4: c-Myc inhibitor; AalphaC: amino-alpha-carboline; KX2-391 and Dasatinib: Src inhibitor; RA: retinoic acid; ATRA: a retinoid

Table 5 Alteration in gene expression by drug treatment

Effect/stimuli	HepG2	Hep3B	References
Upregulation of AFP by HBX	Yes	No	Arima et al. (2002)
Upregulation of p21 by AG490	No	Yes	Kusaba et al. (2007)
Upregulation of NOX4 by TGF-β	No	Yes	Carmona-Cuenca et al. (2008)
Upregulation of FAS by bleomycin	Yes	No	Muller et al. (1997)
Upregulation of Bax by AG490	No	Yes	Kusaba et al. (2007)
Upregulation of MMP-3 by PGI/AMF	Yes	No	Yu et al. (2004)
Activation of caspase-3 by NK cells	No	Yes	Kim et al. (2004)
Stimulation of APP synthesis by OM	Yes	No	Richards et al. (1992)
Stimulation of EPO by IL-6	No	Yes	Jelkmann et al. (1994), Wenger et al. (1998)
Induction of LDLR by PMA	50-fold	10-fold	Wilson et al. (1997)
Lysozyme production by IL-6	Decrease	Increase	Kobsel and Ramadori (1994)
Induction of CYP24A1 by $1,25(OH)_2D_3$	5300-fold	0	Horvath et al. (2012)

PGI/AMF: phosphoglucose isomerase/autocrine motility factor; APP: acute phase proteins; OM: oncostatin M; EPO: erythropoietin; LDLR: low density lipoprotein receptor; PMA: phorbo1-12-myristate-13-acetate; $1,25(OH)_2D_3$: 1,25-dihydroxyvitamin D_3

cell growth is inhibited to a greater degree in Hep3B than in HepG2 cells (Wiesenauer et al. 2004). Moreover, treatment with DOX increases the level of active, phosphorylated ERK and causes apoptosis in cell lines. Treating HepG2 cells with a combination of DOX + U0126 effectively suppresses ERK phosphorylation induced by DOX and increases growth inhibition by enhancing apoptosis. In contrast, the same treatment does not enhance apoptosis or the inhibition of cell proliferation in Hep3B cells despite the level of phosphorylated ERK is decreased (Choi et al. 2008). Furthermore, vitamin K2 (VK2) activates ERK phosphorylation and inhibits cell growth in Hep3B but not in HepG2 cells (Matsumoto et al. 2006). Taken together, the differences in response to drug treatments of HepG2 and Hep3B cells may well be attributable to the RAS/RAF/MEK/ERK pathway.

JAK/STAT pathway

JAK/STAT pathway plays an important role in cellular processes like differentiation, proliferation, and apoptosis in liver cancer (Huynh 2010). STATs are latent in the cytoplasm and become activated through tyrosine phosphorylation which typically occurs through JAKs or growth factor receptor tyrosine kinases. Activated STATs translocate into nucleus and serve as transcription factors for multiple downstream target genes. In normal cells, ligand-dependent activation of STATs is transient, but in liver cancer, STAT proteins are often constitutively activated in primary tumors (Calvisi et al. 2006; Kusaba et al. 2007; Mair et al. 2011). This constitutive activation is because of the inactivation of specific STAT inhibitors, SOCSs (suppressors of cytokine signaling) which are inactivated by DNA hypermethylation in liver and other cancers (Yoshikawa et al. 2001; Niwa et al. 2005). Thus, dysfunction of the STAT inhibitors such as cytokine-inducible SH2-protein (CIS), SOCS-1, SOCS-2, SOCS-3, and SHP1 (SH2-containing phosphatases) has been shown to account for the constitutive activation of STAT-3 and -5 in liver cancer. The different levels of SOCS-1 as shown in Table 1, and perhaps other components in the pathway may determine the different chemoresistance of HepG2 and Hep3B to drug treatments. AG490 is a JAK2 specific inhibitor that inhibits the phosphorylation of STAT3 (Fuke et al. 2007; Kusaba et al. 2007). Treatment with AG490 induces cell cycle arrest in HepG2 cells but obvious apoptosis in Hep3B cells (Kusaba et al. 2007). Similarly, SRC (short for sarcoma, a proto-oncogene encoding a tyrosine kinase) family members are also involved in the constitutive activation of STAT3 in various malignances, including liver cancer (Kusaba et al. 2007; Lau et al. 2009). Therefore, treatment with SRC specific inhibitors KX2-391 and Dasatinib causes different responses between HepG2 and Hep3B cells as shown in Table 4 (Lau et al. 2009). Hence, JAK/STAT pathway is also involved in the different response of HepG2 and Hep3B cells to drug treatment.

Hedgehog pathway

The same holds true for the Hh pathway. Hh pathway plays an important role in diverse cellular development processes, including embryonic patterning, cell differentiation and organ morphogenesis (Theunissen and de Sauvage 2009; Ho and Alman 2010). It is frequently activated by mutations of the components in this pathway in multiple cancers, including liver cancer (Chang et al. 2006). Aberrant activation of Hh signaling is associated with cellular growth, survival and adult stem cell maintenance (Ho and Alman 2010). There are three mammalian Hh proteins: Sonic Hedgehog (SHH), Indian Hedgehog (IHH) and Desert Hedgehog (DHH) and three commonly studied downstream targets: PTCH1, SMO and GLI1 (Theunissen and de Sauvage 2009). This pathway is activated when SHH, IHH or DHH binds to their receptor, PTCH1. When unoccupied by ligand (SHH, IHH or DHH), PTCH1 acts as a tumor suppressor by binding to and repressing SMO, which is a proto-oncoprotein to activate transcription factors, such as GLI1 (Theunissen and de Sauvage 2009). Therefore, specific inhibition of the components in Hh pathway might be a therapeutic option for tumor treatment. Indeed, Hep3B cells respond to treatments with SMO antagonists, KAAD-cyclopamines or SHH neutralizing antibodies, which specifically inhibit the Hh signaling pathway, with suppressed cell growth and enhanced apoptosis. In contrast, HepG2 cells are resistant to the same treatments (Chang et al. 2006; Sicklick et al. 2006). Again, the different levels of SHH as shown in Table 1 (Chang et al. 2006; Sicklick et al. 2006) and other components (Huang et al. 2006) in Hh pathway may account for the differential sensitivity to the specific inhibition of Hh pathway between HepG2 and Hep3B cells.

Underlying mechanisms responsible for the differences between HepG2 and Hep3B cells

HBx is associated with but may not be responsible for the majority of genes expressed differently in HepG2 and Hep3B cells

In order to identify determinants of the differences between HepG2 and Hep3B, we first examined the factors that regulate the differentially expressed genes in these two cell lines. As indicated in Table 1, 11 out of the 19 differentially expressed genes are regulated in Hep3B cells by the presence of HBV, especially by the HBV x protein (HBx). HBx is a multifunctional regulator and plays a crucial role in HCC development. Its functions have been comprehensively reviewed in recent review articles (Tsai and Chung 2010; Ng and Lee 2011; Tan 2011) regarding gene transcription modulation, signal transduction pathways, cell cycle progression, cell death, protein degradation and genetic stability. As shown in Table 1 and discussed in the aforementioned reviews, all the three signal pathways known to respond differently to drug treatments as described in the previous section are regulated by HBx (Ng and Lee 2011; Arzumanyan et al. 2012). Hence, HBx is sometimes considered to be a viral oncoprotein (Koike 2009; Neuveut et al. 2010) and could be the chief regulator of the differential gene expression between HepG2 and Hep3B because it regulates the majority of those genes listed in Table 1. In order to determine whether the HBx is really responsible for the differential expression of genes in HepG2 and Hep3B cells, we examined the reported alteration of gene expression in HBx knockdown studies. Surprisingly, we found that none of the genes listed in Table 1 has been reported to be either up- or down-regulated in the human cells (Xie et al. 2011) or in mouse model (Fukuhara et al. 2012) when HBx is knocked down. This finding seems to suggest that the differential expression of genes between HepG2 and Hep3B is not simply dependent on HBx. Moreover, the expression of either TGF-β type I receptor or SOCS-1 has been shown to be independent of HBx (Shih et al. 2000; Bock et al. 2008). Therefore, HBx may not be the critical factor in governing the differential expression between HepG2 and Hep3B cells.

p53 may not be associated with the differences in HepG2 and Hep3B cells

To survey whether other factors are involved in governing the differential expression of genes between HepG2 and Hep3B cells, we focused on the connection of p53 with the expression of these genes because the transcription factor p53 is the most extensively and intensively studied tumor suppressor gene. p53 is activated in response to virtually all stress signals, including drug treatment to induce DNA damage and oncogene activation (Lane et al. 2010). The normal p53 activity is the most frequently altered in tumors (Vazquez et al. 2008; Lane et al. 2010). Furthermore, in the remaining tumors with wild-type p53, it is often inactivated through other mechanisms. Consequently, the almost universal loss of p53 activity in cancers has been spurring enormous efforts to develop p53-targeted cancer therapeutics as well as attempt to connect various experimental observations with p53 (Fuster et al. 2007; Vazquez et al. 2008; Lau et al. 2009). Because HepG2 cells are known to contain wild-type p53 and Hep3B cells are p53 deficient, it is, therefore, natural to ask whether p53 plays a critical role in the observed differences in gene expression and in response to drug treatments between the p53 wild-type containing HepG2 cells and the p53-deficient Hep3B cells. This question can be answered by comparing the drug treatment effects on HepG2 cells after p53 knockdown and/or on Hep3B cells after p53 restoration by overexpression. In p53 knockdown studies using cDNA microarray assays to obtain a global analysis of endogenous p53 target genes, it has been shown that only Fas/Apo-1 and TGFβ type I receptor (Goldstein et al. 2012), cyclin D1 (Lefort et al. 2007), or none (Chau et al. 2009) is responsive to p53 knockdown.

Furthermore, in order to determine whether the differential drug responses between HepG2 and Hep3B cells are dependent on the status of p53 or not, we analyzed the published data of differential responses against drug treatments in HepG2 and Hep3B cells as shown in Table 2. The different effects of drug treatment include cell cycle arrest at G1 or G2 stages, and apoptosis in both HepG2 and Hep3B cells, and the dependence of the effects on p53 status in HepG2 cells. Among the 19 reports available, we found that 17 of them do not give direct evidence of p53 dependence of the differential drug responses in HepG2 and Hep3B cells, the other two reports give direct but contradictory evidence regarding the dependence on p53 of the differential drug responses in HepG2 and Hep3B cells. The inconclusive or even contradictory findings could simply be due to the fact that the p53-dependence has not been well examined by experiments. But it may also be possible or even more likely that the differential drug responses between HepG2 and Hep3B cell are not dependent on p53 status at all, hence, no publishable positive results have been obtained.

Based on the above discussion, we postulate that p53 may not be the most critical factor to contribute to determining the differential gene expression and responses to pharmacological and therapeutic treatment between HepG2 and Hep3B cell lines. Instead, these differences between these two cell lines may well be due to their different origins.

The different origins of biopsy specimens may be responsible for the differences between HepG2 and Hep3B

HepG2 and Hep3B cell lines are derived from different sources of biopsy specimens. HepG2 was isolated from liver biopsy specimens of primary HB, whereas Hep3B was from primary HCC (Aden et al. 1979; Knowles et al. 1980). According to a hierarchical hepatic cellular lineage model for liver cell maturation and liver cancer development, HB is derived from neonatal liver stem cells with more potential and HCC is arisen from more differentiated mature hepatocytes in liver lobule (Sell and Leffert 2008). Hence, HepG2 and Hep3B come from different differentiation stages in liver cell lineage, which may well explain the differences between them.

Furthermore, a recent comprehensive proteome profiling study (Slany et al. 2010) has shown that in cytoplasmic proteome, HepG2 retains much more hepatocyte-related features, while Hep3B, more fibroblasts-related features and more mesenchymal proteins indicative for epithelial to mesenchymal transition (EMT). In secretome, HepG2 cells express a proteome profile with a quite considerable number of and amount of plasma proteins characteristic for hepatocytes, and Hep3B cells contain few and relatively small amounts of these proteins. Moreover, Hep3B cells secrete five proteins that are characteristic for mesenchymal cells and may be marker proteins for EMT, notably, the follistatin-related protein 1, which is usually found in mesenchymal cells, such as fibroblasts. Therefore, it has been suggested that HepG2 is closer to hepatocyte, whereas Hep3B to fibroblast (Slany et al. 2010). Apparently, hepatocytes and fibroblasts are developmentally unrelated and differ in various functional and phenotypic characteristics, but are often experimentally exchangeable. Mouse fibroblasts can be induced directly to hepatocyte-like (iHep) cells by overexpression of a Forkhead box A protein (Foxa), either alone or in combination with GATA-binding protein 4 (Gata4) without the need for liver progenitor stage (Huang et al. 2011; Sekiya and Suzuki 2011). However, an intermediate EMT stage is needed to induce fibroblasts from hepatocytes by treatment of TGF-beta in liver fibrosis (Zeisberg et al. 2007). Therefore, it seems that Hep3B cells are hepatocytes with EMT, but HepG2 cells are hepatocytes without EMT. EMT is a rapid and reversible change of phenotype of the cells from polarized and epithelial to fibroblastoid and mesenchymal cells (Saulnier et al. 2010). It has an important role in the development of many tissues during embryogenesis, but can also be recapitulated during pathological progression, such as fibrosis and tumorogenesis, including in HCC. EMT may also occur during the transformation, immortalization or in vitro culture of hepatocytes (Slany et al. 2010). Multiple factors such as miRNAs, epigenetic modifications as well as transcriptional factors have been identified to be involved in EMT (Thiery and Sleeman 2006; De Craene and Berx 2013), and differences of these factors may also account for the differences between HepG2 and Hep3B cell lines.

Implications

As a conventional practice to draw scientifically sound and more generalized conclusions, scientists in cancer research and drug development usually use more than one cell line in their assessments. In liver cancer, HepG2 and Hep3B cell lines sharing similarities represent a mainstay of liver cancer biology. Therefore, they are frequently employed in experimental manipulations, including drug treatment, mechanistic studies, and various high-throughput applications. However, different or inconsistent outcomes, which we summarized in Tables 1, 2, 3, 4 and 5 in this review, have resulted from comparative analyses of these two cell lines. Covering the main differences, this review would be of interest to the community of both clinical and basic scientists in both cancer research and drug development, in particular to those beginners entering these fields.

HBx has been widely recognized to be essential for viral replication and to contribute to hepatocarcinogenesis. It regulates the expression of many genes involved in various life processes, therefore, HBV infection, in particular the expression of HBx has been suggested to contribute to the differential gene expression and hence account for differences in drug responses and signal transduction between HepG2 and Hep3B. Similarly, transcriptional factor p53 plays a critical role in directly or indirectly regulating a large number of target genes in genomic stability, cell cycle, senescence, apoptosis, DNA repair and recombination. Like in many other studies, we attempted to link the differences in other gene expression and drug responses between HepG2 and Hep3B cells to differential expression levels of p53 between the two cell lines. However, based on our analyses, neither HBx nor p53 is found to be the crucial determinant of the differences between HepG2 and Hep3B.

Instead of HBx or p53 alone, multiple factors associated with the phenotypic characteristics of the two cell lines from different origins are more likely to be responsible for the differences between HepG2 and Hep3B cell lines. Phenotypically, HepG2 is more hepatocytic and Hep3B is more fibroblastic with EMT from hepatocytes. According to a hierarchical cell lineage model, HB (HepG2) is derived from neonatal liver stem cells with more potential, and HCC (Hep3B), from more differentiated mature hepatocytes in liver lobule. Therefore, the substantial differences between HepG2 and Hep3B could be accounted for by the differences of the maturation of liver cancer cells and complex process of EMT due to the different origins of biopsy specimens, rather than by a single “master molecule” or “hub molecule”, if it exists.

Conclusions

In conclusion, HepG2 and Hep3B are two liver cancer cell lines with a wide range of differences in ethnic origins, biology, genetics and biochemistry. These differences may lead to differential responses to pharmacological agents in these two cell lines, including different signaling responses to specific inhibitors, varied chemosensitivity to therapeutic drugs, as well as diverse effects on cell cycle progression induced by the different treatments. Based on the evidence available, we propose that the difference in the origins of biopsy specimens and associated multiple factors, rather than a single protein such as HBx or p53, may be the critical contributors to govern these differences between HepG2 and Hep3B cell lines.

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