The Hepatocyte and the Cancer Cell: Dr Jekyll and Mr Hyde

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INTRODUCTION

The liver plays a major role in metabolism, especially in the detoxification of diverse groups of substrates, both endogenous compounds and xenobiotics. Hepatic uptake of these compounds from sinusoidal blood is accomplished by transporter proteins localized in the basolateral membrane of hepatocytes, whereas hepatic efflux of bile acids, metabolites, and/or drugs is mediated by adenosine triphosphate (ATP)-binding cassette (ABC) transporters (Figure 66.1; reviewed by Alrefai and Gill [1]).

The strange case of Dr Jekyll and Mr Hyde

Despite the use of numerous treatment modalities and chemotherapeutic agents, the survival rate for individuals with HCC has not improved during the past few decades. Although the reasons for this failure are multifactorial, intrinsic resistance to chemotherapy [2–4] and/or antiviral treatment [5, 6] rank as primary. While both types of resistance deserve equal consideration and can sometimes be explained by the same mechanisms, this chapter will focus on resistance to chemotherapy.

Drug resistance can be attributed to a number of mechanisms, including decreased uptake [7, 8], increased detoxification [9], alteration of target proteins [10–12], or increased efflux [13, 14]. Several of these pathways can lead to MDR, in which the cell becomes resistant to several drugs in addition to the initial compound administered [15]. The multidrug-resistant cancer cell often displays other properties, such as genome instability [16, 17], polymorphisms in cytochrome P450 (CYP) [18], and loss of checkpoint control [19], which complicate further therapy (Figure 66.2).

Hepatocytes, in some ways, exhibit the “split personality” described in the story of Dr Jekyll and Mr Hyde. Their beneficial, healing function of filtering out harmful compounds can be transformed into the inevitable failure of chemotherapeutic treatment. The similarity in gene expression profiles of normal hepatocytes and multidrug-resistant cells is striking. Once a neoplasm develops, the particular gene expression pattern renders it well equipped to resist chemotherapeutic treatments. ABC transporters are key players in MDR and are well represented in liver cells. Since the discovery of the first ABC transporter gene (ABCB1) in 1976 [20, 21], huge amounts of data have been collected, highlighting the complexity of the mechanisms involved in MDR. However, considering the number of cancer patients who still experience treatment failure due to this phenomenon, it seems that we are still in the initial phase of understanding MDR.
In this chapter, we will first review what we have learned in the last few decades about the genetic profiles of hepatic tumors, with emphasis on the SLC uptake transporters and ABC transporters, two of the main contributors to MDR. In the second part, we will stress recent data indicating the critical role of polymorphisms in treatment success.

**Role of transporters and phase I enzymes in hepatocytes (See also Chapters 21, 23, 24 and 43)**

**Hepatic uptake transport: the solute carriers**

Uptake transport in hepatocytes is mediated by transporters belonging to the solute carrier (SLC) family, which includes approximately 360 transporters classified in 45 gene families (classification of the SLC superfamily is outlined at http://www.bioparadigms.org/slc/menu.asp). Genes of the SLC superfamily encode passive transporters, ion-coupled transporters, and exchangers (reviewed by Hediger et al. [22]).

In the liver, organic anion transporting polypeptides (OATPs) encoded by the solute carrier organic anion-transporting polypeptide (SLCO) gene family, organic anion transporters (OATs) encoded by the SLC22 gene family, organic cation transporters (OCTs) also encoded by the SLC22 gene family, and Na\(^+\)-taurocholate cotransporting polypeptides (NTCPs) encoded by SLC10A1 are involved in the transport of many kinds of endogenous compounds and drugs (reviewed by Shitara et al. [23]). Na\(^+\)-dependent bile acid uptake is mediated by NTCP, whereas Na\(^+\)-independent bile acid uptake is mediated by OATPs [24].
Figure 66.2 Multidrug resistance mechanisms in cancer cells. Following drug treatment, several mechanisms result in resistance to either a small number of related drugs or to a broad range of structurally and functionally unrelated drugs, which is known as MDR. The first response to drug treatment is the activation of signal transduction pathways through integrins, growth factors (GFs), Wnt/FZD, and sonic hedgehog (SHH/PTCH), which activate genes such as Bcl2, blocking apoptosis, and glucosylceramide synthase (GCS), affecting membrane lipids. Cancer cells also harbor mutations (*) that impair cell cycle checkpoints (e.g. p53, pRB) and phase I and II enzymes, resulting in increased expression of DNA repair genes and increased drug detoxification. Epigenetic modifications generally occur after drug treatment through activation of genes including HP1 and HDAC. Lastly, transporters play a critical role in MDR. While efflux pumps (i.e. ABC transporters, ABCs) are involved in drug compartmentalization, several of these transporters are often found to be over-expressed in the cell membrane, whereas uptake transporters (i.e. solute carriers, SLCs) are down-regulated. GFRs, growth factor receptors; Wnt, wingless; FZD, frizzled receptor; SHH, sonic hedgehog; PTC, patched; CER, ceramide; MAPK, mitogen-activated protein kinase; PI3K, phosphatidyl inositol 3′-kinase; TFs, transcription factors; HDAC, histone deacetylases; HP1, heterochromatin protein 1

In the OATP family, OATP1B1 (also known as liver-specific organic anion transporter-1 (LST-1)/OATP-2/OATP-C, which is encoded by SLC01B1), OATP1B3 (also known as LST-2/OATP-8, which is encoded by SLC01B3), and OATP2B1 (also known as OATP-B, which is encoded by SLC02B1) are expressed in the liver (reviewed in and [25, 26]). OATP1B1 mediates the uptake of not only bile acids but also conjugated steroids, thyroid hormones, eicosanoids, and some drugs, including 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor, pravastatin, and the anticancer drug, methotrexate [27]. OATP1B3 has a characteristic expression pattern: less expression in normal hepatic cells (compared with that of OATP1B1) and higher expression in various human cancer tissues and in different tumor cell lines derived from the stomach, colon, pancreas, liver, gall bladder, and lung [28]. The substrate specificity of OATP1B3 is similar to that of OATP1B1, and includes methotrexate. The expression pattern and substrate specificity of OATP2B1 are different from those of OATP1B1 and 1B3. Although OATP2B1 is mainly expressed in the liver, it is distributed in a wide range of organs such as the spleen, placenta, lung, kidney, heart, ovary, small intestine, and brain. OATP2B1 does not transport bile acid but transports sulfobromophthalein, estrone-3-sulfate, and dehydroepiandrosterone sulfate [29].

OCT1 and OAT2 belong to the SLC22 transporter family and mediate the basolateral uptake of organic cations and anions (see [30]). Substrates of OCT1 include serotonin, prostaglandin E2, F2α, and drugs such as acyclovir. OCT1 also mediates uptake of platinum drugs
Hepatic efflux transport: the ABC transporters (See also Chapters 23, 24 and 43)

ABC transporters are responsible for active efflux transport from the hepatocyte to the bile canaliculus through the apical/canalicular membrane and to the sinusoidal blood through the basolateral membrane. Apical membrane transporters include ABCB1/Pgp (P-glycoprotein), ABCB4/MDR3, ABCC11/BSEP (bile-salt export pump), ABCC2/MRP2 (multidrug resistance protein 2), ABCC2/BCRP (breast cancer resistance protein), and the two half transporters ABCG5 and G8. Under physiological conditions, the bile-salt export pump [ATP binding cassette (ABC)B11] transports monovalent bile acids [32, 33], ABCB4 exports phosphatidylcholine [34], ABCB2 mediates the translocation of divalent bile acids through a co-transport mechanism with reduced glutathione (GSH), glucuronate, or sulfate [35] and the ABCG5/G8 heterodimer exports cholesterol [36, 37]. ABCB1 and ABCG2 are involved in the protection of the organism by limiting the absorption of xenobiotics (see [38, 39]). ABCC2 is also known to efflux xenobiotics, leading to resistance to chemotherapeutics [40, 41].

Five members of the ABC (MRP) transporter C subfamily are localized in the basolateral membrane; ABCC1/MPR1, ABCC3/MPR3, ABCC4/MPR4, ABCC5/MPR5, and ABCC6/MPR6. These transporters mediate the excretion of organic anions from hepatocytes to sinusoidal blood. ABCC1 is barely expressed in adult liver cells [42] and is found primarily in intracellular vesicles [43]. This transporter can transport a broad range of organic conjugates, including steroids and bile salt (BS) conjugates [44]. ABCC1/MPR8 shares with ABCC3 the ability to transport monovalent BSs such as cholate, taurocholate, and glycocholate, and conjugated BSs [45]. Although the function of ABCC11 in the liver has not been undisputedly demonstrated, the presence of its transcripts in the liver with the recent determination of its substrates has led to speculation concerning its potential role in bile acid homeostasis [46, 47]. While the affinity of ABCC4 for sulfated BSs and steroids was demonstrated by Zelcer et al. [48], ABCC5 and C6 mediate transport of a wide range of conjugated organic anions. These transporters also have a critical role in multidrug resistance (MDR) (reviewed by Gillet et al. [13] and Szakacs et al. [14]).

Key role of CYP450, Phase I enzymes (See also Chapter 70)

Once inside the cell, compounds are converted to metabolites by CYP enzymes, key players in the mechanisms of detoxification. An up-to-date database and nomenclature for CYP450 enzymes can be found at http://drnelson.utmem.edu/CytochromeP450.html [49]. These phase I metabolism enzymes comprise a superfamily of oxidases responsible for the oxidation of numerous endobiotics and thousands of xenobiotics. Although there are 57 P450 genes in the human genome, only 10 contribute to drug metabolism, with the main contribution coming from three isoforms, CYP3A4, CYP2D6, and CYP2C9 [18]. CYP enzymes metabolize endo- and xenobiotics into reactive species, substrates for phase II enzymes, which transform them into soluble non-toxic metabolite conjugates, further excreted through the bile, and sinusoidal blood. Phase II enzymes are involved in conjugation reactions including glutathionylation [50], glucuronidation [9], and sulfation [51].

In the last decade, studies have highlighted the synergism between CYP enzymes and ABC transporters, which can be considered as phase III in the detoxification system [52]. Many conjugated metabolites are substrates of members of the ABC/MDR subfamily of ABC transporters [44]. This synergism may also occur when metabolites produced by CYP enzymes, especially CYP3A4, are better substrates for ABCB1 than the parent compound or when ABCB1 prolongs the duration of absorption by necessitating a subsequent entry of the compound/drug into the cell [53]. This process increases exposure to CYP enzymes and could prevent kinetic saturation of these proteins [54, 55]. The co-regulation of phase I and II metabolism enzymes via ligand-activated transcription factors, such as the aryl hydrocarbon receptor (AhR), the constitutive androstane receptor (CAR), the pregnane X receptor (PXR), and nuclear factor E2-related factor 2 (Nrf2), has been demonstrated ([52, 56]; see Chapter 22). Recently, Jigorel et al. reported a complex pattern of transporter regulation by xenobiotics in human hepatocytes, where efflux and uptake transporters are synergistically up- and down-regulated following drug administration through activation of ligand-activated transcription factors [8]. The interplay between not only ABCC but also OATP transporters and CYP enzymes was recently reviewed by Nies et al. [57].

Prevalence of liver cancer and challenges associated with treatment

Hepatocellular carcinoma (HCC) accounts for approximately 80–85% of primary liver cancer, whereas intrahepatic cholangiocarcinoma (≈14%) and fibrolamellar carcinoma (≈1%) are the two other types of neoplasm that
occur [58]. Liver cancer is the sixth most common cancer and the third most deadly cancer worldwide, with an overall mortality of ~600,000 deaths per year [58]. In most cases, HCC arises as a consequence of underlying liver disease, usually a viral hepatitis [hepatitis B virus (HBV) or hepatitis C virus (HCV)] [59, 60]. However, cirrhosis from alcohol or non-alcoholic steatohepatitis, hereditary tyrosinemia, and primary hemochromatosis are other pathologies that predispose individuals to the development of HCC [59, 60].

The variety of diseases that give rise to liver cancer render the choice of treatment difficult and challenging; for an excellent review, see Llovet and Bruix [61]. Briefly, surgery is the mainstay treatment for patients with early-stage tumors. Either resection or transplantation is advocated if the HCC is within the Milan criteria [62]. Non-surgical treatments, including percutaneous ethanol injection (PEI) [63], radiofrequency ablation (RFA) [63, 64], and transcatheter arterial chemoembolization (TACE) [65], are used as adjuvant therapy to surgery but also to treat unresectable HCC. Although a meta-analysis showed a beneficial survival effect for patients with intermediate HCC treated by chemoembolization/TACE using doxorubicin and cisplatin, the survival benefit of systemic chemotherapy for the treatment of liver cancer is marginal at best. Indeed, systemic administration of doxorubicin has been evaluated in more than 1000 patients in clinical trials. There is a partial response in only around 10% of the cases, without any evidence of survival advantage [66, 67]. Lastly, a phase III randomized placebo-controlled trial (the SHARP trial) demonstrated that sorafenib, a tyrosine kinase inhibitor, improves survival in patients with advanced HCC. This will likely be established as the first line of therapy for advanced HCC [68, 69]. Further analysis will reveal whether sorafenib contributes alone or in combination with other chemotherapy to a decrease in the morbidity of liver cancer.

**Lack of consensus on reliable clinical staging systems and gene signatures**

Classification of HCC is hampered by similar issues brought to light in the leukemia and lymphoma field in the early 2000s. Numerous clinical staging systems, such as Barcelona-Clinic Liver-Cancer (BCLC) [77], the French model Groupe d’Etude de Traitement du Carcinoma Hepatocellulaire (GRETCH) [78], and Japan Integrated Staging (JIS) [79], have been suggested to predict patient survival with HCC, but none of these models predicts outcome accurately. Although some studies have claimed that the BCLC staging systems are superior [80–82], the current literature is conflicting. Indeed, while some studies did not report the superiority of any of the current clinical staging models [83], others supported one staging system over the others [84–87]. These conflicting reports can be explained to some extent by the complexity of the pathology and lack of molecular information in the models used.

Data have been generated in the last 8 years in an attempt to unravel the molecular pathogenesis of HCC (see Chapters 60, 61). Laurent-Puig *et al.* highlighted two groups of patients with good and poor prognosis based on chromosomal stability status and genetic mutations [88]. More recently, this group reported an unsupervised study of more than 100 HCC samples that identify six groups of patients according to transcription patterns, chromosomal stability, promoter methylation status, and genetic mutation analysis [89]. Ye *et al.* reported a supervised study that allowed classification of patients with metastatic HCCs and highlighted genes such as *osteopontin* as highly correlated with metastasis and poor patient survival [90]. The same researchers recently published a 20-microRNA (miRNA) signature that may assist in identifying patients with HCC who are likely to develop metastases [91]. Another miRNA-based study reported a 19-miRNA signature in HCC associated with patient survival from cirrhosis and hepatitis [92]. Lee *et al.* tested the prognostic value of gene signatures obtained from 91 HCC samples. Those tumors were sub-classified into two groups strongly associated with patient survival [93]. Recently, Wurmbach *et al.* analyzed 75 samples representing the stepwise carcinogenic process from preneoplastic lesions (cirrhosis and dysplasia) to HCC, including neoplastic stages (very early HCC to metastatic

**IMPACT OF GENE EXPRESSION PROFILING ON MOLECULAR CHARACTERIZATION OF HEPATOCELLULAR CARCINOMA**

The challenge of clinical oncology has been to target specific therapies to well-defined distinct cancer types to maximize efficacy and minimize toxicity of treatment. Significant advances in treatment are usually spurred on by technological developments. In this case, high-density DNA microarrays have revolutionized clinical oncology, unraveling the heterogeneity of cancers, and therefore refining the classification of many cancers that had previously been diagnosed on the basis of staging systems that did not incorporate biological information concerning the tumor [70–72]. A striking example was reported by Golub *et al.*, who demonstrated the feasibility of cancer classification based solely on gene expression [73]. They distinguished between acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) without previous knowledge of these classes [73]. A number of studies subsequently confirmed the valuable contribution of genome-wide analyses of various tumors, including HCC (reviewed by Dunphy [74], Thorgeirsson *et al.* [75], and Quackenbush [76]).
Integrative systems biology needed for classification and diagnosis

Systems biology has the potential to refine the classification of HCC and improve diagnosis and prognosis (reviewed by Vivekanandan and Singh [97]). However, the success of such an integrative approach depends on our ability to “speak the same language.” Indeed, the variety of experimental systems developed to characterize cancers at a molecular level, such as complementary DNA (cDNA) expression profiling, comparative genomic hybridization (array CGH), promoter arrays, SNP arrays, and so on, has increased dramatically in the last 5 years. In addition, the variety of platforms proposed for each of those analytical systems is remarkable. The multitude of normalization processes proposed also renders an integrative computational and analytical approach extremely challenging [98]. A combined database would also serve as an analytical tool that could compute the degree of overlap between global signatures and specific signatures imported by a researcher, comparing his or her findings with standardized data [98]. Rhodes et al. developed Oncomine, a bioinformatics initiative aimed at collecting, standardizing, analyzing, and delivering cancer transcriptome data to the scientific community [99]. The first version of this database was released in 2003 and contained 40 microarray data sets and nearly 100 differential expression analyses, allowing users to query differential expression results for a gene of interest across collected data sets [100]. The current version compiles 18 000 cancer gene expression experiments, and automated analysis has identified gene networks activated and repressed in human cancers (http://www.oncomine.org) [99]. In addition, they further developed the concept of molecular signature maps by demonstrating their utility in generating hypotheses that link cancer types and subtypes, pathways, mechanisms, and drugs [101]. Similar resources have arisen focusing specifically on HCC. They include EHCO (Encyclopedia of Hepatocellular Carcinoma Genes Online), a repository of genes found relevant in HCC [102], and OncoDB.HCC, an integrated oncogenomic database of HCC [103]. For additional information, the reader is directed to the websites http://ehco.iis.sinica.edu.tw/ and http://oncodb.hcc.ibms.sinica.edu.tw/index.htm.

A considerable amount of data has been generated that has significantly improved our understanding of HCC pathobiology. We are now at a crossroads, where we need integrative approaches to extract biological insights efficiently from vast datasets generated on genomic, proteomic and metabolomic levels. As discussed in Chapters 60, 61 [104], this approach will further help not only to stratify patients into clinically homogeneous groups, but also to uncover the origins of tumor cells and distinct pathways involved in the molecular pathogenesis of HCC.

ROLE OF MULTIDRUG RESISTANCE IN THE INTRACTABILITY OF HCC

As mentioned earlier, the survival benefit of systemic chemotherapy in the treatment of liver cancer is only marginal at best. The reason for such a high level of resistance to drug treatment has been solely explained by the over-expression of ABCB1/MDR1, an ABC transporter cloned in 1987 [105]. Since that time, a vast amount of data has been generated, bolstering our understanding of ABC transporter functions in tumor resistance and concerning the multifactorial nature of those mechanisms (see Figure 66.2; recently reviewed by Mimeault et al. [106]). With regard to the liver, an MDR-centered study, in the strict sense, has not been performed. Instead, uptake and efflux transporters have been studied for their pivotal role in hepatobiliary elimination through ADMET (absorption, distribution, metabolism, excretion, and toxicity) profiles of drug candidates involved in the drug discovery process.

In this section, we stress the role of two transporter superfamilies in MDR mechanisms. We briefly review the emerging role of SLCs, drug uptake transporters, in MDR mechanisms and their role in clinical drug resistance. We also review the current knowledge on the involvement of ABC transporters in those mechanisms.

The role of solute carriers (SLCs) in multidrug resistance (see also chapter 21)

Cellular entry represents the first step in the mechanism of action of anticancer agents. Transporter-related MDR could result from not only increased efflux of drugs mediated by ABC transporters but also reduced drug uptake. The net accumulation of an anticancer drug in a cell is probably influenced by the concurrent actions of uptake and efflux transporters. Relative differences in uptake and efflux might contribute to drug resistance and be a primary factor in the differential response of various tumor types to the drugs. In cancer cells, studies have shown that some uptake transporters belonging to SLC families confer sensitivity to anticancer drugs [28, 107–112].

OATP1B3, which is expressed predominantly at the basolateral (sinusoidal) surface of hepatocytes, and has
been shown to be expressed in various human cancer tissues and also in different tumor cell lines including HCC, confers sensitivity to methotrexate in cancer cells [107]. Kullak-Ublick and co-workers reported the differential expression of OATP1B3 and OATP1B1 in HCC [113]. They showed inhibition of OATP1B3 expression in HCCs that over-express hepatocyte nuclear factor 3β (HNF3β), one of several liver-enriched transcription factors shown to be differentially expressed in HCC compared with non-tumor liver tissue.

Some studies have shown that OCT1, OCT2, and OCT3 mediate cell sensitivity to platinum drugs such as cisplatin, carboplatin, and oxaliplatin [31, 109–111]. OCT1 was also shown to mediate the uptake of imatinib [108]. A clinical study conducted by Crossman et al. showed that the expression level of OCT1 messenger RNA (mRNA) prior to treatment with imatinib in non-responders was only one-eighth of that seen in responders [114]. Dose escalation of imatinib could overcome resistance to standard-dose therapy in patients with chronic myeloid leukemia (CML), as reported by Kantarjian et al., which indicates that intracellular concentration of imatinib might be crucial for the therapy of CML [115].

Knowing whether these uptake transporters are expressed and functional in specific cancers can be a powerful tool to predict response to specific therapies. Okabe et al. used a bioinformatic approach to identify SLC substrates [112], mRNA expression of 28 members of the SLCO and SLC22 family in 60 diverse cancer cell lines (the NCI-60) used by the National Cancer Institute (NCI) to screen for anticancer activity was profiled. By correlating expression profiles with growth inhibitory profiles of 1429 compounds (including anticancer drugs and drug candidates) tested against the cells, it was confirmed that OCTN1/SLC22A4 confers sensitivity to doxorubicin in cancer cells [112]. Unfortunately, cell lines derived from HCC are not included in the NCI-60 panel. Further study is needed to elucidate the gene and/or protein expression of SLCs in the HCC cell lines and clinical samples.

ATP-binding cassette (ABC) transporters mediate multidrug resistance

The ABC transporter encoding genes are widely dispersed in the genome and show a high degree of sequence identity among eukaryotes. For extensive reviews, see Gillet et al. [13] and Szakacs et al. [14]. The ABC family includes 48 members divided into seven sub-families. The nomenclature for human ABC transporter genes is provided at the website http://nutrigene.4t.com/humanabc.htm.

In the late 1990s, tumor resistance to therapy was correlated with expression of three ABC transporters, namely ABCB1/MDR1 [20, 105], ABCC1/MRP1 [116], and ABCG2 [117]. For recent reviews, see Callaghan et al. [38], Deeley et al. [44], and Polgar et al. [39]. To date, 13 ABC transporters (ABCA2, ABCB1, ABCB4, ABCB11, ABCC1–6, ABCC11–12, and ABCG2) were associated with drug resistance. For reviews, see Gillet et al. [13] and Szakacs et al. [14].

The determination of the expression profiles of ABC transporter genes in multidrug-resistant cell lines has opened up new avenues for the diagnosis of MDR in the clinic and for monitoring expression profiles in clinical biopsies and their correlation to clinical treatment. Four microarray-based assays for the detection of ABC transporter genes have been developed [118–121]. Annerau et al. developed a high-density microarray platform containing probes specifically matching 36 ABC transporters and also 70-mer oligonucleotides, allowing the analysis of 18 000 unique human genes [118]. Huang et al. developed a similar oligonucleotide microarray [120]. However, large amounts (25 and 12.5 μg, respectively) of total RNA were required for reverse transcription to run each array. Gillet et al. developed a low-density DNA microarray for profiling the expression of 38 ABC transporter genes which required less total RNA [119]. Finally, Liu et al. designed a semi-quantitative assay to detect the expression of 47 ABC transporter genes [121]. This last approach has two main drawbacks: it is not reliable for the detection of moderate changes in expression levels, and is not applicable for quantitative detection of abundant mRNAs [121]. These technologies require relatively large amounts of sample and often have poor probe specificity for individual transporters with highly homologous gene family members, such as those involved in MDR.

Quantitative real-time reverse transcriptase polymerase chain reaction (qRT-PCR) has emerged as a fast and sensitive detection method that allows reproducible quantification of very low amounts of total RNA. Two approaches have been used to quantify 47 human ABC transporters in tissue samples and tumor cell lines. The first approach, developed by Langmann et al. [122], was based on qRT-PCR using Taqman chemistry, whereas Szakacs et al. measured ABC gene expression levels by qRT-PCR using SYBR Green [123]. Although these techniques are more reliable and accurate than microarrays, they are tedious and require multiple pipetting steps, which can introduce variability. Langmann et al. recently developed an assay to quantify 47 human ABC transporters using Taqman chemistry in a high-throughput platform termed Taqman low-density array [124]. These studies suggest that more than 25 ABC transporters can be involved in chemotherapy-induced resistance [119, 123, 125–127].

The role played by ABC transporter genes in clinical treatment and tumor recurrence is a subject of debate. This is well illustrated by experimental and clinical studies related to drug-mediated resistance of leukemia patients. Leukemia has been used as a model disease in some studies (reviewed by Ross [128], van den Heuvel-Eibrink et al. [129], and Hirose et al. [130]). In contrast to adult AML, for which the role of ABCB1 gene expression in
the drug resistance of tumors and prognosis of patients is widely accepted [131, 132], the data for adult ALL are conflicting [132–134]. Therefore, ABC transporters other than ABCB1 may have an effect on treatment response and prognosis of adult ALL. The contribution of the ABCC1, ABCC3, and ABCG2 transporters to treatment outcome of adult ALL patients has been shown by some, but not all, authors [135, 136], leaving the prognostic relevance of these ABC transporters open to discussion. In solid tumors, most attention has been directed to the role played by the ABC transporter proteins in MDR observed in breast cancers, particularly by ABCB1, ABCC1, and ABCG2. However, it is difficult to define their exact role in the clinical drug resistance observed in this disease [137, 138]. ABCG2 has been detected in at least one drug-resistant breast tumor cell line [117]. Like ABCB1 and C1, its relevance to clinical drug resistance in breast tumor is still disputed [139–141] (reviewed by Polgar et al. [39] and Robey et al. [142]).

Several ABC transporters expressed in tumors that have not yet been linked to drug resistance could have prognostic relevance. This view is supported by data published by Vitale et al. exploring the expression of ABC transporters associated with antigen processing (TAP), namely TAP1 (ABCB2) and TAP2 (ABCB3) [143]. In a collection of five specimens of normal mammary tissue and 53 primary breast carcinoma lesions, TAP1 and TAP2 expression was significantly associated with tumor grading. Like normal mammary tissue, the low-grade (G1) breast carcinoma lesions showed strong staining for TAP1 and TAP2. In contrast, only a few of the high-grade (G2 and G3) breast carcinoma lesions displayed a normal expression pattern. These data demonstrate an association of human leukocyte antigen (HLA) class I antigen and TAP down-regulation with tumor progression in breast carcinoma, and suggest that loss of TAP may represent a mechanism employed by cancers to escape the host’s immune pressure or may reflect accumulation of abnormalities associated with neoplastic progression. Accumulation of defects in antigen processing and presentation may be responsible for reduced recognition of malignant cells by putative clinically relevant tumor-specific T cells. Recent data obtained on ABCB5 suggested that this transporter could be a marker of melanoma cancer-initiating cells [144]. Cancer-initiating cells, also known as cancer stem cells, might underlie the intractable nature of many human cancers, explaining why conventional cancer therapy fails in many patients. For a review, see Alkatout et al. [145]. Although the concept is exciting, knowledge of ABCB5 from the genomic to the proteomic level is rudimentary, and does not support this hypothesis.

Although considerable effort has been made to understand the role of ABC transporters in clinical samples, attempts to transform those transporters into clinical targets have been unsuccessful. However, expression of many ABC transporters in HCC is at levels sufficient to confer drug resistance and may contribute to resistance without being the limiting determinants, especially given the extraordinary variety of drug-resistance genes expressed in HCC.

**IMPORTANCE OF PHARMACOGENETICS IN MULTIDRUG RESISTANCE**

The introduction of pharmacogenetics to the clinic is now proposed to individualize therapy [146, 147]. Indeed, inter-individual differences in drug response are major causes of adverse drug reaction and drug treatment failure. Drug bioavailability is dependent on an individual’s expression of drug transporters, such as ABC transporters or uptake transporters. Individual variations in drug plasma level are also, in part, explained by identification of single nucleotide polymorphisms (SNPs) revealing distinct phenotypes of drug-metabolizing enzymes.

**Genetic variation in ABC transporters**

A number of recent reports have addressed genetic polymorphisms in drug transporters; for an excellent review, see Cascorbi [148]. Among the 48 ABC transporters, ABCB1 is one of the best studied and characterized, with more than 50 SNPs reported [148–150]. Hoffmeyer et al. were the first to show association of the synonymous SNP 3435C>T in exon 26 with decreased expression of ABCB1 in the duodenum of patients with the T allele (variant) compared with those with the C allele (wild-type) [151]. However, a subsequent study reported that this effect might be due to the non-synonymous SNP 2677G>T/A [152], which is frequently linked to C3435T. In contrast to these earlier reports, Gerloff et al. reported no changes in digoxin clearance between Caucasian patients carrying the variant or the wild-type allele [153]. Elevated ABCB1 expression was reported in Japanese and Caucasian patients carrying the variant allele (3435T) [154, 155]. The non-synonymous SNP 2677G>T/A is also subject to conflicting data [156].

Recently Kimchi-Sarfaty et al. analyzed the role of synonymous mutations in protein folding and function [10]. Synonymous SNPs (3435C>T, 1236C>T) and non-synonymous 2677G>T in the ABCB1 gene sequence result in a protein with altered drug and inhibitor interactions, without a change in expression levels, possibly due to altered protein folding related to a change in the rhythm of translation [10]. Based on our current knowledge, overall drug bioavailability is only moderately influenced by ABCB1 polymorphisms, as compared with variants of the drug-metabolizing enzymes (CYP family) [157, 158]. Although the findings for ABCC1, showing...
a high rate of polymorphisms, are similar to those for ABCB1, studies on ABCG2 report a significant effect of polymorphisms on ABCG2 expression and function [147, 150, 159].

The correlation of ABC transporter genetic variants with treatment outcomes is gradually being clarified, yet the overall picture is still puzzling, as much of the published data are conflicting. Nevertheless, many studies reporting correlation between SNPs and clinical outcome indicate the necessity to pursue further investigations. Initiatives such as the Pharmacogenetics Research Network could aid the development of this complex field. One of their various goals is to understand how genetic variation in membrane transporters contributes to variation in drug transport [160].

Genetic variation in solute carriers

Among the SLCs, the OCTs, OATs, and OATPs have been proposed to have critical roles in the absorption, distribution, and excretion of xenobiotics and endogenous compounds in the liver, kidney, central nervous system, intestine, and other tissues. Genetic polymorphisms of these transporters may alter drug pharmacokinetics, triggering interindividual differences in the safety and efficacy of drugs. OCTN1, encoded by SLCO1B1, is expressed abundantly in the liver and mediates the hepatic uptake of a broad range of organic ions, has been well studied concerning SNPs. For an excellent review, see Niemi [161]. Certain SNPs (e.g. \( \text{SLCO1B1-388A} > \text{G}, \text{521T} > \text{C}, \text{578T} > \text{G} \)) have been shown to affect its surface expression and/or function in vitro and/or in vivo in humans. However, no data exist on the consequences of \( \text{SLCO1B1} \) variants in MDR.

A number of sequence variants have been reported in OATP1B3 (\( \text{SLCO1B3} \)), also expressed in the liver and in cancers. Letscher et al. demonstrated that \( \text{SLCO1B3-1564G} > \text{T} \), encoding OATP1B3-G522C, abolished the transport of bile acid but not other substrates [162]. OATP1B3 was shown to mediate uptake of paclitaxel. Investigation of the functional consequences of mutation in \( \text{SLCO1B3} \) showed that paclitaxel pharmacokinetics were not associated with \( \text{SLCO1B3-344T} > \text{G} \) or \( \text{699G} > \text{A} \) [163].

OCTN1, encoded by \( \text{SLC22A4} \), is expressed mainly in the kidney and is thought to be a transporter responsible for renal disposition of cationic compounds. An SNP of \( \text{SLC22A4} \) that produces the amino acid mutation L503F is associated with risk for developing Crohn’s disease [164]. The effects of the 503F variant on \( \text{SLC22A4} \) specificity may diminish uptake of physiological compounds while increasing uptake of potential toxins. One intronic SNP in \( \text{SLC22A4} \) also showed strong association with rheumatoid arthritis, which, like Crohn’s disease, has a pathogenesis associated with inflammation and autoimmunity [165].

\( \text{SLC22A4} \) is expressed in various cancer cell lines and mediates uptake of the anticancer agent doxorubicin [112].

As described above, the OCT1 (\( \text{SLC22A1} \)), OCT2 (\( \text{SLC22A2} \)), and OCT3 (\( \text{SLC22A3} \)) transporters may mediate uptake of some platinum anticancer drugs [31, 109–111]. Although genetic variants of \( \text{SLC22A1} \) and \( \text{SLC22A2} \) showed altered transport of some of their substrates, such as metformin, which is used in therapy for type 2 diabetes mellitus [166, 167], their transport of platinum anticancer drugs has not been investigated.

Unlike ABC transporters, SLCs have not been intensively focused on as candidate transporters for anticancer drugs. There is little information about the association between MDR and SNPs in the SLCs.

CONCLUSION – DR JEKYLL OR MR HYDE?

In the Introduction to this chapter, we characterized hepatocytes through an analogy to the story of Dr Jekyll and Mr Hyde. Do these cells really have a “split personality?” We have learned a great deal in the last decade with the development of high-throughput genomic profiling systems and unraveling of the human genome sequence. These breakthroughs have provided new insights in cancer research, revolutionizing current classification of almost all cancers and their clinical management.

HCC is a complex and heterogeneous disease. Genomic expression profiling has focused on the predictive power of gene signatures for overall survival (see Chapters 60, 61 and [88, 89, 93]). Attention has also been directed towards gene signatures associated with the carcinogenic process, from preneoplastic lesions to neoplastic stages, including very early HCC to metastatic tumors exemplified by findings reported by the Llovet group [94].

The field of ABC transporter-mediated drug resistance has also been revolutionized by technological and scientific advances. From 13 ABC transporters known to be involved in MDR, now more than 25 transporters are suggested to be involved in tumor resistance. However, a red flag has been raised regarding the study of gene families with a high degree of homology, such as ABC transporter genes. The lack of specificity and sensitivity of platforms used to profile expression of ABC transporters is most likely one reason for observed discrepancies. In work in progress in our laboratory [168], we are evaluating three unique gene-expression profiling technologies to ascertain which technology provides the best tool for drug discovery and has potential for clinical applications in personalized medicine. We determined that Taqman low-density arrays (TLDAs) are the most sensitive and selective in measuring ABC transporter gene-expression patterns in a group of intensively studied cancer cell lines.

An additional major concern raised in this chapter is standardization of reported gene signatures. How can
Figure 66.3 Systems biology model for HCC study. This model proposes four starting points: blood samples, tumor biopsies, normal liver, and in vivo–in vitro models. Technological advances allow the study of all the cellular levels from the genomic to the metabolomic through various types of microarrays, 2D electrophoresis, mass spectrometry, and so on. The challenge is now the development and expansion of integrative tools that will help to extract thoroughly all the insights of those studies. The capsules in bold type indicate the key research objectives of the studies scientists communicate with each other without a common language? In this regard, leading research institutions have the duty to promote standardization of the data reported. A database such as Oncomine, a bioinformatics initiative aimed at collecting, standardizing, analyzing, and delivering cancer transcriptome data to the scientific community, is invaluable. Considerable data have been compiled on multiple biological levels. We need now to develop tools allowing integrative approaches to extract biological insights efficiently from vast datasets generated at the genomic, proteomic, and metabolomic levels.

There is a relative paucity of research on the intractability of HCC from the perspective of MDR. Can the malignant transformation of liver cells be accompanied by multifactorial MDR gene expression? Does the genetic background of hepatocytes predispose them to transformation (i.e. in the role of Mr Hyde), or are those genes irrelevant to the pathobiology of HCC, protecting the organism (i.e. in the role of Dr Jekyll)? Further investigation is needed to answer these questions. Figure 66.3 proposes a systems biology model for further analysis of HCC based on what is currently in the literature and the authors’ vision of multidimensional biology now possible in the post-genomic era.

REFERENCES


