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Anticancer Drug Metabolism: Chemotherapy Resistance and New Therapeutic Approaches

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1. Introduction

Over the last decades, several studies have demonstrated that cancer cells have a unique metabolism compared to normal cells (Herling et al., 2011). Metabolic changes occurring in cancer cells are considered to be fundamental for the transformation of normal cells into cancer cells and are also responsible for the resistance to different types of chemotherapeutic drugs (Cree, 2011). Therefore, resistance to chemotherapy represents a major problem in the treatment of several tumor types. Among the different metabolic and signalling pathways that are altered in cancer cells, variations in the expression and activity of several drug-metabolizing enzymes play a critical role in drug resistance (Rochat, 2009). Resistance can occur prior to drug treatment (primary or innate resistance) or may develop over time following exposure to the drug (acquired resistance). In some patients, prolonged exposure to a single chemotherapeutic agent may lead to the development of resistance to multiple other structurally unrelated compounds, known as cross resistance or multidrug resistance.

Cancer cell metabolism is also closely linked to molecular oxygen concentration. Indeed, weak blood irrigation is frequently encountered in solid tumors and is responsible for hypoxic environment which is associated with invasive/aggressive phenotype and therapeutic resistance (Shannon et al., 2003). Hypoxia also contributes to drug resistance because some chemotherapeutic drugs require oxygen to generate free radicals that contribute to toxicity. Moreover, hypoxia might modulate expression of enzymes directly involved in metabolism of chemotherapeutic drugs, thereby limiting the toxic effects of these drugs on cancer cells. On the other hand, new therapeutic strategies aim at using bioreductive drugs that are selectively toxic to hypoxic cells (McKeown et al., 2007).

The proposal of this chapter is to describe the role of anticancer drug metabolism in chemotherapy resistance but also its importance for the development of new approaches, taking advantage of the specificity of cancer cells metabolism.

2. Anticancer drugs

Anticancer or chemotherapy drugs are powerful chemicals that kill cancer cells by arresting their growth at one or more checkpoints in their cell cycle. Their main role is thus to reduce

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and prevent the growth and spread of cancer cells. However, because anticancer agents rapidly affect dividing cells, normal cells are also affected. This is especially true in tissue with high cell turnover such as the gastrointestinal tract, bone marrow, skin, hair roots, nails... Consequently, side effects are commonly observed with various types of chemotherapies. More than 100 different drugs are used today for chemotherapy, either alone or in combination with other treatments.

For several years, the most effective drugs used in cancer chemotherapy were DNA-damaging agents (Gurova, 2009). These drugs can be divided into different categories based on their mechanism of action. Inhibitors of DNA synthesis inhibit essential biosynthetic processes or are incorporated into macromolecules (DNA and RNA). These drugs are either structural analogues for heterocyclic bases or agents interfering with folate metabolism (heterocyclic bases and folic acid are DNA building blocks) and they inhibit main steps in the formation of purine and pyrimidine bases as well as nucleotides (Parker, 2009). This class of agent includes antifolates (methotrexate, pemetrexed) (Goldman et al., 2010), antipyrimidines (5-fluorouracil, capecitabine, eniluracile, hydroxyurea) (Longley et al., 2003) and antipurines (6-mercaptopurine, 6-thioguanine). Another class of drugs directly damages DNA by adding methyl or other alkyl groups onto nucleotide bases (Izbicka and Tolcher, 2004). This in turn inhibits their correct utilization by base pairing leading to mutation, DNA fragmentation as well as inhibition of DNA replication and transcription. These anticancer drugs include alkylating agents (cyclophosphamide, ifosfamide, melphalan, chlorambucil), platinum-based drugs (cisplatin, carboplatin), antibiotics (anthracyclines, dactinomycin, bleomycin, adriamycin, etoposide) and topoisomerase II inhibitors (camptothecine, irinotecan, topotecan). Molecules belonging to the third class affect synthesis or breakdown of the mitotic spindle (Risinger et al., 2009). These drugs disrupt the cell division by either inhibiting the tubulin polymerization and therefore the formation of the mitotic spindle (vinblastine, vincristine) or by stabilizing microtubules (paclitaxel, docetaxel).

Over the past 20 years, the elucidation of different signal-transduction networks that are responsible for neoplastic transformation has led to rationally designed anticancer drugs that target specific molecular events. These targeted cancer drug candidates include protein kinase inhibitors that represent an important and still emerging class of therapeutic agents. Clinically approved kinase-targeted oncology agents include 1) small molecules such as imatinib (targeting Abl, Platelet-Derived Growth Factor Receptor (PDGFR)), gefitinib and erlotinib (targeting epidermal growth factor Receptor (EGFR)), sorafenib (targeting PDGFR, EGFR, Raf-1, c-kit) or 2) antibodies such as Cetuximab or Bevacizumab that inhibit EGFR and vascular endothelial growth factor receptor (VEGFR), respectively (Sebolt-Leopold and English, 2006). Unfortunately, these new targeted drugs also face major obstacles similar to those that challenge traditional agents.

3. Anticancer drug metabolism and resistance

3.1 Anticancer drug metabolism

In vivo, after absorption in the organism, xenobiotics (including anticancer drugs) are typically metabolized through a number of parallel and/or sequential reactions. Metabolism occurred through two distinct consecutive phases named “phase I” and “phase II”, although this order is not exclusive (phase I not always followed by phase II; phase II not always preceded by phase I) (Iyanagi, 2007). Phase I reactions are most commonly described as
“functionalization” reactions and include oxidations, reductions, and hydrolysis (Guengerich, 2007, 2008). These reactions introduce a new polar functional group to the parent drug (oxidation), modify an existing functional group in order to be more polar (reduction) or unmask existing polar functional group (hydrolysis). The most common functional groups exposed or introduced in the phase I reactions are hydroxyl (-OH), amino (-NH₂), and carboxylic acid (-COOH).

Phase II reactions are most commonly described as conjugation reactions and include glucuronidation, sulfonation, glycine/glutamine conjugation, acetylation, methylation, and glutathione (GSH) conjugation (Bock et al., 1987; Jancova et al., 2010). Conjugations allow linking a new group either to the parent drug or to phase I metabolites. Some conjugations cause a dramatic increase in the polarity and thus favor excretion of a drug by adding an ionized functional group: sulfonation, glucuronidation, and amino acid conjugation. Other conjugation reactions are just likely to cause termination of therapeutic activity: methylation and acetylation. GSH conjugation reaction protects against reactive metabolites.

In this chapter, we will be interested mainly by two distinct families of enzymes, cytochrome P450s (CYP) and glutathione transferases (GST) belonging to phase I and phase II metabolism, respectively.

3.1.1 Cytochrome P450s

CYP enzymes are key players in the phase I-dependent metabolism, mostly catalyse oxidations of drugs and other xenobiotics. More than 57 active human CYP genes and 58 pseudogenes have been described (Sim and Ingelman-Sundberg, 2010). Most of these genes are polymorphic and more than 434 different alleles of genes encoding CYP enzymes have been identified. The CYP3A (CYP belonging to family 3, subfamily A) enzymes are involved in the metabolism of about 50% of all drugs currently on the market (Bu, 2006). CYPs also participate in the metabolic activation of several carcinogens such as aflatoxin B₁ (Langouet et al., 1995). As a result of the CYP-dependent metabolism, intermediates that exert toxicity or carcinogenicity can be formed. In most cases, these metabolites are targets for phase II enzyme dependent reactions, rendering them inactive polar products suitable for excretion via the kidneys. Concerning anticancer agents, CYPs are involved not only in cytotoxic drugs detoxication but also in the activation of prodrugs making them therapeutically effective (McFadyen et al., 2004). Prodrugs are inactive agents that are converted to active cytotoxic drugs upon exposure to tumor tissues exhibiting high expression of activating enzymes. This targeting strategy minimizes toxicity towards normal tissues while it increases delivery of active agent to the tumor tissue. Cyclophosphamide, ifosfamide, dacarbazine, procarbazine, tegafur, and thiotepa are metabolized by CYPs in the liver and this activation reaction is required for therapeutic activity (Rodriguez-Antona and Ingelman-Sundberg, 2006). Another example is 1,4-bis-((2-(dimethylamino-N-oxide)ethyl)amino)5,8-dihydroxy anthracene-9,10-dione (AQ4N), a bioreductive prodrug that needs activation by CYP2S1 and CYP2W1 in tumor tissues to be converted to a topoisomerase II inhibitor (Nishida et al., 2010). Therefore, because CYPs are involved in either the bioactivation or the inactivation of both carcinogens and anticancer drugs (Huttunen et al., 2008), they play important roles in the etiology of cancer diseases and as determinants of cancer therapy (Oyama et al., 2004).
3.1.2 Glutathione transferases

GSTs are a family of ubiquitous intracellular enzymes that catalyze the conjugation of GSH to many exogenous and endogenous compounds (Hayes et al., 2005). These include chemical carcinogens, therapeutic drugs and products of oxidative stress. In addition to their major role in catalyzing the conjugation of electrophilic substrates to GSH, these enzymes have GSH-dependent peroxidase (Hurst et al., 1998) and isomerase (Johansson and Mannervik, 2001) activities. GSTs play an important role in the protection against reactive molecules such as electrophilic xenobiotics (anticancer drugs, pollutants or carcinogens) or endogenous alpha,beta-unsaturated aldehydes, quinones, epoxides, and hydroperoxides formed as secondary metabolites during oxidative stress. Over the last decade, different studies have demonstrated that GSTs also have a non-catalytic function via their interaction with some kinases (Adler et al., 1999; Cho et al., 2001; Gilot et al., 2002) or other proteins (Dulhunty et al., 2001; Wu et al., 2006) thus playing critical roles in stress response, apoptosis and proliferation. GSTs are members of at least three gene families: the cytosolic (or soluble) GSTs that are divided in seven families: alpha, mu, pi, theta, sigma, zeta and omega (Hayes et al., 2005); the mitochondrial GST (kappa class) (Morel and Aninat, 2011) and the membrane-associated proteins involved in eicosanoid and glutathione metabolism (MAPEG) (Jakobsson et al., 2000; Jakobsson et al., 1999). The cancer chemotherapeutic agents adriamycin, 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), busulfan, carmustine, chlorambucil, cisplatin, crotonoyxymethyl-2-cyclohexenone, cyclophosphamide, melphalan, mitozantrone and thiotepa are potent substrates of GSTs (Hamilton et al., 2003; Hayes and Pulford, 1995; Lien et al., 2002). Metabolism of these anticancer drugs by GSTs is related to several drug resistance phenomena and adverse toxicity effects (Townsend and Tew, 2003b).

3.1.3 Drug transporters

Drug passage across biological membranes is possible through two different mechanisms. The first one involves passive trans-cellular transport and concerned lipophilic molecules. The second one depends on carrier-mediated transporters, among which, we distinguish those requiring ATP-dependent hydrolysis as the first step in catalysis (ABC transporters such as multidrug resistance protein (MDR), multidrug resistance-associated protein (MRP), and breast cancer resistance protein (BCRP)) from those driven by an exchange or co-transport of intracellular and/or extracellular ions with the substrate (organic anion transporter (OAT), organic anion-transporting polypeptide (OATP), sodium taurocholate co-transporting peptide (NTCP), organic cation transporter (OCT), novel organic cation transporter (OCTN) and oligopeptide transporter (PEPT)) (Keppler, 2011; Li et al., 2010; Ni et al., 2010; Svoboda et al., 2011).

Active transporters are of great interest to pharmacologists since they are responsible for both the uptake and the efflux of drugs and are key elements of the pharmacokinetic characteristics of a drug (Degorter et al., 2011). Indeed, it has now become clear that transporters are essential for the uptake, accumulation, distribution and efflux of drugs. For example, drug efflux transporters including the P-glycoprotein pump (Pgp), the multidrug-resistant protein-1 (MRP1) and the BCRP actively pump drugs such as chemotherapeutics out of the cells, thereby reducing their intracellular accumulation and making the cell insensitive to different drugs such as anthracyclines, vinca-alkaloids or taxanes. Among the major known ABC transporters,
ABCB1 gene, also known as *MDR1*, encoding Pgp is by far the best characterized and understood efflux transporter (Goda et al., 2009; Wu et al., 2011). It is predominantly expressed in several tissues including the luminal surface of intestinal epithelia, the renal proximal tubule, the bile canalicular membrane of hepatocytes and the blood brain barrier (Ho and Kim, 2005). Pgp plays an important role in limiting intestinal drug absorption and brain penetration as well as in facilitating renal or biliary excretion of drugs. The MRPs are involved in the drug efflux from the liver or kidney into the peripheral blood (e.g. MRP1, MRP3, and MRP6), or from the liver, kidney and small intestines into the bile, urine and intestinal lumen respectively (MRP2) (Keppler, 2011). Since GSH-, glucuronide-, sulfate-conjugates and organic anions such as methotrexate, indinavir, cisplatin, vincristine and etoposide are all MRP substrates, MRPs are also crucial in human drug disposition and toxicity.

### 3.2 Anticancer drug resistance

The development of chemotherapy resistance remains a major problem to the effective treatment of many tumor types. Resistance can occur prior to drug treatment (primary or innate resistance) or may develop over time following exposure (acquired resistance). In some patients, prolonged exposure to a single chemotherapeutic agent may lead to the development of resistance to multiple other structurally unrelated compounds. This process is known as cross resistance or multidrug resistance (MDR). In primary resistance, MDR can occur without prior exposure to chemotherapy. Several mechanisms, including alterations in drug pharmacokinetic and metabolism, modification of drug target expression or function, drug compartmentalization in cellular organelles, altered repair of drug-induced DNA damages, changes in apoptotic signaling pathways or expression of proteins directly affecting cellular drug transport are responsible of anticancer drug resistance (Figure 1).

![Fig. 1. Representation of different mechanisms involved in anticancer drug resistance. ↑: increase; ↓: decrease; OATP: organic anion-transporting polypeptide; OCT: organic cation transporter; Pgp: P-glycoprotein; MRP: multidrug resistance associated proteins; CYP: cytochrome P-450; SOD: superoxide dismutase; GST: glutathione transferase; MAPK: mitogen activated protein kinase.](www.intechopen.com)
3.2.1 Drug transport

Drug transporters are the key determinants for the uptake, accumulation, distribution and efflux of several chemotherapeutic drugs. Interestingly, overexpression of these drug transporters in tumors has been demonstrated by several studies. Pgp is expressed in approximately 40% of all breast carcinomas (Trock et al., 1997), although another study reported values as high as 66% (Larkin et al., 2004). MRP3 was found to be the predominant MRP isoform in gallbladder carcinomas and cholangiocellular carcinomas and the intrinsic multidrug resistance in these carcinomas seems to be dependent on the expression of MRP3 (Rau et al., 2008). The MRP4 (also named cMOAT or ABCC4) gene is overexpressed in cisplatin resistant human cancer cell lines with decreased drug accumulation (Taniguchi et al., 1996). Platinum-resistant tumor cells are capable of eliminating platinum GSH-conjugates in an ATP-dependent manner through an active efflux mechanism mediated by a GS-X pumps (Ishikawa et al., 2000; Suzuki et al., 2001). MRP8, encoded by ABCC11 gene, is able to confer resistance to fluoropyrimidines by mediating the MgATP-dependent transport of the cytotoxic metabolite 5'-fluoro-2'-deoxyuridine monophosphate (Guo et al., 2003). MRP2 expression has been suggested to affect the efficacy of cisplatin treatment in patients with hepatocellular carcinoma (Korita et al., 2010). Overexpression of these pumps in tumor cells gives them the ability to evade the treatment by drugs such as cisplatin, fluoropyrimidines, doxorubicin and etoposide in different types of cancer (Jedlitschky et al., 1996; Kool et al., 1997; Xu et al., 2010; Zelcer et al., 2001). Therefore, the use of chemomodulators to inhibit efflux transport has been tested in an attempt to overcome this resistance (Baumert and Hilgeroth, 2009; Zhou et al., 2008). In this way, a recent study has demonstrated that indomethacin and SC236 inhibit Pgp and MRP1 expression and thus enhance the cytotoxicity of doxorubicin in human hepatocellular carcinoma cells (Ye et al., 2011).

3.2.2 Drug inactivation/detoxification

Drug-metabolizing enzymes can also play an important role in reducing the intracellular concentration of drugs and in affecting cancer drug resistance. Interestingly, certain drugs require to be metabolized by these enzymes before exerting their cytotoxic effects. The expression of drug-metabolizing enzymes can therefore either potentiate or reduce the toxicity of chemicals and variations in both the activation and the inactivation pathways are important variables that can lead to drug resistance. In model systems, it appears that both oxidation (phase I) and conjugation (phase II) enzymes play critical roles in protecting cells against many drugs and thus play a key role in drug resistance.

3.2.2.1 Involvement of cytochrome P450s

As previously mentioned, CYPs are involved in both activation and detoxication of xenobiotics, including therapeutic drugs. CYP3A4 plays an important role in the metabolism of several anticancer agents (e.g. taxanes, vinca-alkaloids and new drugs such as imatinib, sorafenib and gefitinib). CYP3A4 metabolizes docetaxel to inactive hydroxylated derivatives. Therefore, a high CYP3A4 activity would result in a poor therapeutic outcome of the drug. Accordingly, in cancer patients treated with docetaxel in combination with the potent CYP3A4 inhibitor ketoconazole, a 49% decrease in docetaxel clearance was found (Engels et al., 2004). A low expression of CYP3A4 in breast tumors resulted in a better
response to docetaxel (Miyoshi et al., 2005). Similarly, hepatic CYP3A4 activity measured by the erythromycin breath test and midazolam clearance predicted docetaxel clearance and demonstrated a higher toxicity in patients with the lowest CYP3A4 activity (Goh et al., 2002). Similarly to docetaxel, irinotecan is inactivated by CYP3A4 and induction of CYP3A4 in patients receiving irinotecan results in a significant decrease in the formation of the toxic metabolite of this drug (Mathijsen et al., 2002). Additionally, CYP3A4 phenotype, as assessed by midazolam clearance, is significantly associated with irinotecan pharmacokinetic (Mathijsen et al., 2004). More recently, a study suggested that the Pregnane X-Receptor (PXR) pathway is also involved in irinotecan resistance in colon cancer cell line via the upregulation of drug-metabolizing genes such as CYP3A4 (Basseville et al., 2011).

Other CYP families also participate to anticancer drugs metabolism. For example, CYP2C19 and CYP2B6 are involved in the activation of the chemotherapeutic agent cyclophosphamide (Helsby et al., 2010) and reduced expression of these CYPs is a potential mechanism of resistance. An interesting study showed a mechanism of acquired resistance to anticancer therapy based on the induction of CYP2C8 and MDR1. In this study, Caco-2 cells were capable of increasing the expression of CYP2C8 as a response to long-term exposure to paclitaxel (Garcia-Martin et al., 2006). Furthermore, the correlation between CYP polymorphism and anticancer drug response has been demonstrated for CYP2B6. Indeed, CYP2B6*2, CYP2B6*8, CYP2B6*9, CYP2B6*4 variant alleles are associated with response to doxorubicin-cyclophosphamide therapy in the treatment of breast cancer and with a worse outcome (Bray et al., 2010). In another study, it has been demonstrated that CYP1B1 inactivates docetaxel and showed that the overexpression of CYP1B1 in a Chinese hamster ovary fibroblast cell line (V79MZ) was correlated to a significantly decreased sensitivity towards docetaxel (McFadyen et al., 2001a; McFadyen et al., 2001b). Finally, other authors suggested that CYP1B1 does not directly inactivate docetaxel but promotes cell survival by another unknown mechanism (Martinez et al., 2008).

Altogether, these studies demonstrate that altered levels of expression or inhibition of CYPs can have profound effects on the sensitivity of target cell to toxic compounds.

### 3.2.2.2 Involvement of glutathione transferases

GSTs are involved in the development of resistance to anticancer drugs by different ways. Indeed, they play a role in the metabolism of a diverse array of cancer chemotherapeutic agents including Adriamycin, BCNU, busulfan, carmustine, chlorambucil, cisplatin, cyclophosphamide, ethacrynic acid, melphalan or thiophospha (Chen and Waxman, 1994; Dirven et al., 1994; Paumi et al., 2001). The roles of GSTs in the metabolism of these anticancer drugs and the correlation between GST expression levels and drug sensitivity have been demonstrated in several studies. For example, the inhibition of GST Pi 1 (GSTM1) expression, through antisense cDNA, has been shown to increase the tumor sensitivity to Adriamycin, cisplatin, melphalan and etoposide (Ban et al., 1996). By contrast, the overexpression of GSTP1 in human renal UOK130 tumor cells was accompanied by a decreased sensitivity to cisplatin, melphalan and chlorambucil (Wang et al., 2007). Similarly, overexpression of Alpha class GST has been correlated with the resistance to alkylating agents in Colo 320HSR cells (Xie et al., 2005) and to doxorubicin in MCF-7 human breast cancer and small cell lung cancer (H69) cell lines (Sharma et al., 2006; Wang et al., 1999).
Overexpression of Mu class GST has been associated with chlorambucil resistance in human ovarian carcinoma cell line (Horton et al., 1999) and with poor prognosis in childhood acute lymphoblastic leukaemia (Hall et al., 1994).

Interestingly, high levels of GSTs are linked either with drug resistance or cancer incidence. GSTP1 has retained much attention because many tumors and cancer cell lines are characterized by high GSTP1 expression. Moreover, increased expression of GSTP1 has been associated to acquired resistance to cancer drugs (Tew, 1994). It is noteworthy that several studies have demonstrated that altered GST catalytic activities caused by genetic polymorphisms are linked to cancer susceptibility and prognosis (McIlwain et al., 2006). For example, GST genotypes are associated with primary and post-chemotherapy tumor histology in testicular germ cell tumors (Kraggerud et al., 2009); GST polymorphisms may have a role in treatment response and osteosarcoma progression (Salinas-Souza et al., 2010) and null genotypes of GSTM1 and GSTT1 contribute to hepatocellular carcinoma risk (Wang et al., 2010).

The non-catalytic functions of GST might also play a key role in the anticancer drug sensitivity. Indeed, the direct interaction and inhibition of various MAP Kinases by GSTs have been demonstrated. These MAP kinases are involved in cell proliferation and apoptosis but also in anticancer drug responses. In the last decade, several studies have demonstrated that GSTs are involved in the control of apoptosis through the inhibition of the Jun N-terminal Kinase (JNK) signaling pathway. Indeed, JNK is inactive and sequestered into a GSTP1-JNK complex (Adler et al., 1999) whereas Apoptosis Signal Kinase 1 (ASK1) and Mitogen-activated protein kinase kinase kinase (MEKK1) interact with GSTM1 leading to their inactivation (Gilot et al., 2002; Ryoo et al., 2004). Thus, the overexpression of GSTs in many tumors or their up-regulation by drugs could represent another mechanism of drug resistance, independent of their enzymatic activity. As an example, cisplatin, chlorambucil, doxorubicin, 5-fluorouracil and carboplatin are among anticancer drugs whose toxicity require the activation of JNK and resistance to these drugs is highly associated to overexpression of GSTs in tumors (Townsend and Tew, 2003a). Therefore, development of GST inhibitors that could prevent MAPK inhibition is considered as a promising strategy to achieve new anticancer drugs in order to increase chemotherapeutic efficiency.

### 3.2.3 Involvement of nuclear transcription factors in drug resistance

Nuclear receptors are a superfamily of transcription factors with 48 distinct members identified within the human genome (Germain et al., 2006). In addition to the classic steroidal hormone receptors, other nuclear receptors act as metabolic sensors that respond to compounds of dietary origin, intermediates in metabolic pathways, drugs and other environmental factors, integrating homeostatic control over many metabolic processes (Sonoda et al., 2008). For example, some aspects of drug metabolism and transport are regulated by pregnane X receptor (PXR) and constitutive androstane receptor (CAR); energy and glucose metabolism are regulated in part by peroxisome proliferator-activated receptor gamma (PPARγ); fatty acid, triglyceride and lipoprotein metabolisms are controlled by PPARα, δ, and γ; reverse cholesterol transport and cholesterol absorption depends on liver X receptor (LXR) activation and bile acid metabolism is regulated by farnesoid X receptor (FXR) (Evans, 2005; Francis et al., 2003).
PXR and CAR are master xenobiotic receptors that regulate the expression of genes involved in drug metabolism and clearance, including drug-metabolizing enzymes and transporters (Evans, 2005). In this part, we will focus on nuclear factors involved in the regulation drug-metabolizing enzymes and drug transporters (PXR, CAR and Nrf2) and on their specific roles in drug resistance.

3.2.3.1 Pregnane X receptor (PXR)

In 1998, a new member of the nuclear hormone receptor family, named PXR (NR1I2), has been identified (Kliewer et al., 1998). PXR is activated primarily by pregnanes and dimerizes with retinoid X receptor (RXR) immediately after its activation by ligand binding. PXR is present in the cytoplasm where it interacts with a protein complex. After its activation, PXR translocates into the nucleus to regulate gene transcription (Squires et al., 2004). PXR recognizes a wide variety of ligands including dexamethasone, rifampicin, spironolactone and pregnenolone 16α-carbonitrile being among the best characterized (Timsit and Negishi, 2007) as well as many anticancer drugs such as microtubule-binding drugs (Raynal et al., 2010). Targets genes of PXR are \textit{CYP3A4}, \textit{MDR1}, \textit{CYP2B6}, members of UGTs superfamily and \textit{MRP3} and \textit{OATP2} transporters (Klaassen and Slitt, 2005; Tolson and Wang, 2010). Due to its capacity to recognize such compounds and to induce transcription of genes involved in the detoxification process, PXR is considered as one of the master regulator of xenobiotic clearance. Moreover, because PXR controls the expression of key genes involved in anticancer drugs disposition, recent works have focused on its potential role in drug resistance (Chen, 2010). The mechanisms of resistance induced by PXR activation probably involve up-regulation of drug-detoxifying enzymes and transporters. Supporting this hypothesis, it has been shown that PXR activation by different ligands induces PXR target genes (\textit{CYP2B6}, \textit{CYP3A4} and \textit{UGT1A1}) and consequently drug resistance in ovarian cancer cells (Gupta et al., 2008). Moreover, PXR induces expression of \textit{CYP3A4} and \textit{MDR1} genes in multiple cell types and the products of these genes are known to detoxify microtubule-binding and topoisomerase-binding drugs. Previous studies have shown that PXR activation regulates Pgp in the blood-brain barrier (Bauer et al., 2004). Interestingly, anticancer drugs such as vincristine, tamoxifen, vinblastine, docetaxel, cyclophosphamide, flutamide, ifosfamide and paclitaxel activate PXR-mediated Pgp induction and thus affect the cytotoxic activity and accumulation of the Pgp substrate rhodamine 123 (Harmsen et al., 2010). Increased expression of PXR leads to higher resistance of HEC-1 cells to paclitaxel and cisplatin (Chen, 2010) and of human colon adenocarcinoma to doxorubicin (Harmsen et al., 2010). In osteocarcinoma, the effectiveness of etoposide was reduced due to activation of PXR and the co-administration of PXR agonists enhanced the clearance of all-trans-retinoic acid (ATRA). This mechanism could potentially contribute to ATRA resistance in the treatment of acute promyelocytic leukemia (APL) and several solid tumors (Wang, T. et al., 2008). However, other mechanisms of resistance (e.g., down-regulation of apoptotic genes) may also play a dominant role (Zhou et al., 2008).

3.2.3.2 Constitutive androstane receptor (CAR)

The constitutive androstane receptor (CAR) is a sister xenobiotic receptor of PXR. CAR was first purified from hepatocytes as a protein bound to the phenobarbital responsive element in the \textit{CYP2B} gene promoter. CAR was subsequently shown to bind to the \textit{CYP2B}
gene promoter as a heterodimer with retinoid X receptor (RXR). Transfected CAR exhibited a high basal activity and was once termed a “constitutively active receptor.” The name of constitutive androstane receptor was conceived due to the binding and inhibition of CAR activity by androstanes (Forman et al., 1998). CAR is retained in the cytoplasm by forming a complex with phosphatase 2A, HSP90 and cytosolic CAR retention protein (Kobayashi et al., 2003). Phenobarbital, 5β-pregnane-3,20-dione, and 5-androstan-3-ol are known CAR ligands (Moore et al., 2000). The hepatomitogen 1,4-Bis[2-(3,5-dichloropyridyloxy)] benzene (TCPOBOP) is a synthetic agonist for murine CAR (Tzameli et al., 2000) and 6-(4-chlorophenyl)imidazo[2,1-b][1,3]thiazole-5-carbaldehydeO-(3,4-dichlorobenzyl)oxime (CITCO) is an imidazothiazole derivative that functions as a selective agonist for human CAR (Ikeda et al., 2005). Upon activation with specific agonist, CAR translocates into the nucleus and binds to the response elements as monomer or CAR/RXR heterodimer. CAR functions as a xenobiotic receptor that participates in the regulation of transcription of drug transporter genes such as MRPs (MRP2, MRP3 and MRP4), OATP2 and MDR1 ((Urquhart et al., 2007). CAR promotes the detoxification and elimination of potentially toxic compounds by modulating the phase I and phase II drug-metabolizing enzymes. Therefore, CAR-mediated expression of xenobiotic-metabolizing enzymes is generally protective, but can be deleterious if toxic metabolites are produced. CAR agonists are able to induce hepatocyte proliferation that depends on c-Myc-FoxM1 function (Blanco-Bose et al., 2008) but also to inhibit Fas-induced hepatocyte apoptosis by depleting the proapoptotic proteins Bak (Bcl-2 antagonistic killer) and Bax (Bcl-2-associated X protein) and increasing the expression of the antiapoptotic effector myeloid cell leukaemia factor-1 (Baskin-Bey et al., 2006).

3.2.3.3 Nuclear factor-erythroid 2p45 (NF-E2)-related factor 2 (NRF2)

The transcription factor Nrf2 (nuclear factor-erythroid 2p45 (NF-E2)-related factor 2) is a major regulator in the basal and inducible expression of various phase II detoxifying and antioxidant enzymes. In the resting state, kelch-like ECH-associated protein 1 (Keap1) functions as an intracellular redox receptor, which binds Nrf2 and targets it for proteosomal degradation. When cells are exposed to oxidative damage, Nrf2 is liberated from Keap1 and translocated into the nucleus where it specifically recognizes an enhancer sequence known as Antioxidant Response Element (ARE). This binding of Nrf2 on ARE sequence results in the activation of redox balancing genes (e.g. heme-oxygenase-1), phase II detoxifying genes (e.g. GSTs and NAD(P)H quinone oxidoreductase-1) and drug transporters (e.g. MRP) (Baird and Dinkova-Kostova, 2011; Taguchi et al., 2011). Several studies have suggested that the activation of Nrf2 protects against chronic diseases such as cardiovascular diseases, neurodegenerative disorders, lung inflammation, fibrosis, diabetes and nephropathy. However, in recent years, the dark side of Nrf2 has emerged and growing evidences suggest that Nrf2 constitutive up-regulation is associated with cancer development, progression and resistance to chemotherapy (Hayes and McMahon, 2006, 2009; Konstantinopoulos et al., 2011; Wang X.J. et al., 2008). Many anticancer drugs are responsible for the production of ROS in cancer cells, a phenomenon which contributes to drug-induced apoptosis. Such species are scavenged by the catalytic activities of superoxide dismutase, catalase, GSH peroxidase, γ-glutamylcysteine synthetase and heme oxygenase-1. These enzymes are members of the ARE-gene battery and are often overexpressed during carcinogenesis and it seems likely that Nrf2 may be responsible for this phenotype.
The down-regulation of Nrf2-dependent response by overexpression of its negative regulator, Keap1, or transient-transfection of Nrf2-siRNA in lung carcinoma, breast adenocarcinoma, neuroblastoma, ovarian cancer and colon cancer rendered cancer cells more susceptible to cisplatin, etoposide, doxorubicin and 5-fluorouracil (Akhdar et al., 2009; Cho et al., 2008; Homma et al., 2009; Wang, X.J. et al., 2008). Induction of nuclear translocation and activation of Nrf2 by 5-fluorouracil, which in turn leads to antioxidant enzymes up-regulation and increases resistance toward cytotoxic effects of this anticancer drug has been recently demonstrated (Akhdar et al., 2009). The inhibition of Nrf2 by a specific flavone, lutolein, leads to negative regulation of the Nrf2/ARE pathway and to the sensitization of human lung carcinoma cells to therapeutic drugs (Tang et al., 2011). KEAP1 gene deletion provoked an aberrant Nrf2 activation and is one of the molecular mechanisms explaining chemotherapeutic resistance against 5-FU in gallbladder cells (Shibata et al., 2008a; Shibata et al., 2008b). Several studies have reported mutations of the interacting domain between Keap1 and Nrf2 leading to a permanent Nrf2 activation in non-small cell lung cancer (Ohta et al., 2008; Padmanabhan et al., 2006). Somatic mutations of the KEAP1 gene were also reported in patients affected by gall bladder tumors and in breast cancer cell line (Nioi and Nguyen, 2007; Shibata et al., 2008a). Although recent studies demonstrated low or no expression of KEAP1 in more than half of non-small cell lung cancers, only two papers investigated the epigenetic alterations of KEAP1 in this type of tumor. An aberrant hypermethylation at the KEAP1 gene promoter in lung cancer cell lines and in five lung cancer tissues has been demonstrated (Wang, R. et al., 2008). More recently, two alterations in KEAP1 gene were detected in one third of the non-small cell lung cancers suggesting that both copies of the gene might be inactivated (Muscarella et al., 2011). In these cases, Keap1 function is impaired, leading to constitutive stabilization of Nrf2 and increased activation of its cytoprotective target genes (Okawa et al., 2006).

All of these findings support the idea that increased Nrf2 expression could facilitate cell growth, survival, resistance to chemotherapy through the activation of cytoprotective factors. Thus, investigating the deregulation of Keap1/Nrf2 pathway may shed light into the understanding of molecular mechanism of chemoresistance.

3.2.3.4 Hypoxia and Hypoxia Inducible Factor-1 (HIF-1)

_Hypoxia and HIF-1α are found in solid tumors_

Around fifty percent of locally advanced solid tumors exhibit hypoxic and/or anoxic tissue areas, heterogeneously distributed within the mass tumors (Vaupel and Mayer, 2007). Consequently, partial pressure of oxygen (PO₂) in tumors is variable and can reach values between 10 to 30 mmHg (equivalent to 1 to 3% O₂), in contrast to a PO₂ of 50–80 mmHg in most normal tissues (Grigoryan et al., 2005). Three converging mechanisms lead to this limited oxygenation in cancer cells. The first one is due to cell proliferation which is responsible for an increase of the tumor mass. The second one is characterized by a loss of structural organization of blood vessels already present or newly formed (angiogenesis) in the solid tumor. This process leads to a decreased irrigation of the tumor. In addition, hematologic status of patients with cancer is frequently modified by the disease itself or by chemotherapy and numbers of them suffer from anemia triggering a reduced oxygen-carrying capacity of the blood (Vaupel and Harrison, 2004; Vaupel and Mayer, 2007). HIF-1 transcription factor is a master regulator of the hypoxic response and HIF-1α subunit is
stabilized during hypoxia. Therefore, overexpression of HIF-1α has been found in many human cancers such as bladder, brain, breast, colon, ovarian, pancreatic, prostate and renal carcinomas (Talks et al., 2000).

**Metabolic adaptation to hypoxia and angiogenesis in solid tumor: HIF-1 and HIF-target genes**

In order to fight against hypoxia, a metabolic adaptation of solid tumors is observed compared to the surrounding normal tissue. This phenomena has been first described by Otto Warburg (Warburg, 1956) fifty years ago. He found that, in contrast to normal tissue where glycolysis is used to produce approximately 10% of ATP (the remaining 90% being obtained by oxidative phosphorylation via the tricarboxylic acid (TCA) cycle); solid tumors produced over 50% of ATP by anaerobic glycolysis, i.e. without oxidative phosphorylation and with lactate production. Interestingly, this phenomenon occurs even if oxygen is available for the mitochondrial function. This altered energy dependency is known as the “Warburg effect” and is a hallmark of cancer cells. Several explanations have been given to understand the use of anaerobic glycolysis rather than oxidative phosphorylation for production of ATP, while this is less efficient for energy production. The first one is linked to the accumulation of mutations in the mitochondrial genome that prevent the proper functioning of mitochondria (Carew and Huang, 2002). As a consequence, oxidative phosphorylation is not enough efficient, forcing the cancer cells to use anaerobic glycolysis for ATP production. The second one involves the activation of a transcription factor specifically activated in cell response to hypoxia: the transcription factor hypoxia-inducible factor-1 (HIF-1).

HIF-1 transcription factor is composed of two protein subunits, HIF-1α and HIF-1β (Wang and Semenza, 1995). Its transcriptional activity depends on the stabilization of HIF-1α. While HIF-1β subunit is constitutively expressed into the cells, expression of HIF-1α protein is thinly regulated at a post-translational level. Hydroxylation of HIF-1α by prolyl hydroxylase domain (PHD) proteins, which target its subsequent proteasomal degradation, is one of the major mechanisms of regulation of HIF-1α cellular levels (Jaakkola et al., 2001). Since the activity of PHD enzymes is inhibited by low oxygen tension, HIF-1α protein is stabilized during hypoxia. As a result, upon hypoxic signal, HIF-1α subunit is stabilized translocated into the nucleus where it binds to HIF-1β to form the active HIF-1 complex. HIF-1 binds to hypoxia-responsive elements (HRE), consensus sequences in the promoter region of more than one hundred genes involved in cell proliferation, differentiation and survival, angiogenesis and energy metabolism that allow the cell, tissue, and organism to adapt to reduced oxygen conditions (Semenza, 2003).

Regarding glycolysis metabolism, several HIF-1 gene targets are directly involved in the switch between aerobic to anaerobic glycolysis. Glucose cell uptake and its metabolism are very active in cancer cells. This high activity is correlated with the induction of expression of both the glucose transporter GLUT1 and the glycolysis enzymes aldolase C and phosphoglycerate kinase 1 (PGK1) (Seagroves et al., 2001; Semenza, 2003). Furthermore, HIF-1 facilitates the conversion of pyruvate into lactic acid by the induction of lactate dehydrogenase A (LDHA) (Firth et al., 1995) and pyruvate dehydrogenase kinase 1 (PDK1) expressions (Kim et al., 2006; Papandreou et al., 2006). PDK1, by inhibiting the activity of the pyruvate dehydrogenase (PDH) (Patel and Korotchkina, 2001), prevents conversion of pyruvate into acetyl-CoA, promotes the conversion of pyruvate into lactate and reduces the
metabolic activities of the TCA cycle and the mitochondrial oxidative phosphorylation. Finally, in order to prevent acidosis due to lactate accumulation, intracellular pH homeostasis is maintained by induction of the expression of the carbonic anhydrase 9 and 12 (CA9 and CA12) (Potter and Harris, 2004), the lactate transporter MCT-4 (Ullah et al., 2006) and the Na+/H+ exchanger NHE1 (Shimoda et al., 2006), all direct gene targets of HIF-1. Thus, those metabolic adaptations confer a selective growth advantage and, combined with angiogenesis, are a prerequisite for metastasis.

HIF-1 also plays a key role in angiogenesis, which is a process describing the growth of new blood vessels (neovascularization) from preexisting vessels. Angiogenesis is critical for tumor development since supply of oxygen and nutrients becomes limited to cancer cells located around 70-100 microns of a blood vessel (Carmeliet and Jain, 2000). Ability of tumor cells to induce angiogenesis occurs by a multi-step process, regulated by many pro-angiogenic factors. One of the strongest stimuli of angiogenesis is hypoxia and its transcription factor HIF-1 (Pugh and Ratcliffe, 2003). Indeed, HIF-1 can directly induce the expression of a number of proangiogenic factors such as the vascular endothelial growth factor (VEGF) and its receptors VEGFR1 and VEGFR2, the angiopoietins (ANG-1 and -2) and their receptors (Tie-1 and Tie-2) and the platelet-derived growth factor PDGF-β (Hickey and Simon, 2006). Of all the pro-angiogenic factors induced by HIF-1, VEGF is the factor that is most expressed in tumors (Dvorak, 2002). In several in vitro and in vivo models, HIF-1 signaling is required for VEGF production and the ability of tumor cells to promote angiogenesis. As such, stem cells HIF-1α-/- injected into nude mice form teratocarcinomas substantially smaller and less vascularized than WT embryonic cells (Ryan et al., 1998).

**HIF-1 and HIF-target genes: Actors for drug resistance**

Hypoxia and HIF-1 contribute to the poor response to anticancer therapy by several mechanisms (Cosse and Michiels, 2008; Tredan et al., 2007; Wouters et al., 2007). Indeed, HIF-1 activation allows expression of a battery of genes involved in survival and cell resistance to chemotherapy. For examples, studies have shown that hypoxia is directly involved in the induction of genes coding for the ABC transporters (MDR1, MRP1 and LRP), responsible for HepG2 cells resistance toward 5-Fluourouracil (Comerford et al., 2002; Zhu et al., 2005). Moreover, a recent study has demonstrated that, by down-regulating the expression of the MAPK-specific phosphatase dual-specificity phosphatase–2 (DUSP2), HIF-1 is involved in the resistance of HeLa and HCT116 cells to cisplatin, oxaliplatin, and paclitaxel (Lin et al., 2011). Hypoxia, by modulating expression of enzymes directly involved in metabolism of chemotherapeutic drugs, such as CYPs, could also limit the toxic effects of these drugs on cancer cells. As such, paclitaxel metabolism into 6α-hydroxypaclitaxel is reduced upon hypoxic conditions compared to normoxic conditions in HepaRG cells (Legende et al., 2009). Furthermore, cytotoxic anticancer drugs require the presence of oxygen to exert their effects via the production of ROS, damaging DNA and inducing cell cycle arrest and death by apoptosis. Therefore, lack of oxygen could interfere for the efficiency of those molecules such as doxorubicin, which exerts its cytotoxic effect by the production of superoxide anion (Grigoryan et al., 2005). Another important point is that solid tumors are often poorly irrigated, leading to a decreased accessibility of anticancer agents to the tumor. Decreased drug concentrations, because of limited drug penetration into tumor masses, participates actively to resistance of the tumor to chemotherapy (Tredan et al., 2007). Finally, hypoxic environment of solid tumors is often correlated with a decrease...
of extracellular pH (acidosis) that also modulates the accumulation and/or cell toxicity of anticancer agents (Gerweck, 1998; Rechert et al., 2002). For example, resistance to mitoxantrone in MCF-7 cells is related to the acidification of extracellular pH (Greijer et al., 2005). Taken together, hypoxia and HIF-1 play a key role in anticancer drug resistance.

3.2.4 Other mechanisms

**Modification of drug target**

Cells survival depends on a balanced assembly and disassembly of the highly conserved cytoskeletal filaments formed from actin and tubulin. Microtubules are assembled from α-tubulin and β-tubulin heterodimers, along with other proteins such as microtubule-associated proteins. Some anticancer drugs (such as vinca-alkaloids) bind to and stabilize free tubulin, causing microtubule depolymerization and others (such as taxanes) bind to and stabilize microtubules, causing a net increase in tubulin polymerization (Zhou and Giannakakou, 2005). These two mechanisms of action inhibit cell division and thereby trigger apoptosis of cells. Altered expression of β-tubulin isotypes (overexpression or mutation) and microtubule-associated proteins is found in many cancer cell lines and xenografts resistant to microtubule inhibitors. These alterations may be associated with the primary or acquired resistance to tubulin-binding agents observed clinically in many tumors (Kamath et al., 2005; Wang and Cabral, 2005). Recently, a novel skeleton microtubule inhibitor, chamaecyparanone C, with anticancer activity triggering caspase 8-Fas/FasL dependent apoptotic pathway in human cancer cells has been identified and its cytotoxicity in a variety human tumor cell lines has been studied (Hsieh et al., 2010). The authors considered that chamaecyparanone C is a promising anticancer compound that has potential for management of various malignancies, particularly for patients with drug resistance.

**DNA repair and cellular damages**

Many anticancer drugs exert their effects by inducing DNA damages. Thus, alterations in enzymes involved in DNA repair can affect drug resistance. Topoisomerase II is a critical enzyme that is involved in DNA replication and repair and reduced topoisomerase II expression or function can contribute to resistance to agents such as anthracyclines (Nitiss, 2009). DNA mismatch repair mediates damage repair from many drugs including alkylating agents, platinum compounds and anthracyclines and this mechanism has been implicated in drug resistance in cancer cells (Bignami et al., 2003).

**Apoptosis**

Resistance can also arise from a failure of the cells to undergo apoptosis following DNA damages or other cellular injuries. Alterations in genes regulating the apoptotic pathway such as BCL2, BCLX (anti-apoptotic proteins) or TP53 promote resistance to anticancer drugs (O’Connor et al., 1997). P53 can trigger elimination of the damaged cells by promoting apoptosis through the induction of pro-apoptotic genes, such as FAS and BAX, and the down-regulation of anti-apoptotic BCL2. Studies have reported that loss of p53 function reduces cellular sensitivity to anticancer drugs. Mutations in the TP53 gene are found in most human breast cancer cell lines, and certain mutations have been linked to de novo resistance to doxorubicin (Aas et al., 1996). On the other hand, the use of adenovirus-mediated TP53 gene therapy reverses resistance of breast cancer cells to adriamycin (Qi et al., 2011).
4. Taking advantage of cancer cell metabolism for drug targeting

4.1 Nuclear factors: Targets for new therapeutic strategies

4.1.1 PXR and CAR

During the last years, several groups have studied the role of PXR antagonists as potential pharmaceuticals for the reversal of drug resistance and enhancement of drug delivery (Biswas et al., 2009; Harmsen et al., 2010). Ketoconazole was originally described as a PXR antagonist (Takeshita et al., 2002). However, significant side effects of ketoconazole were reported mainly because of its off-target effects (e.g., cortisol synthesis, hepatic toxicity), some of which are related to its capacity to inhibit CYP activities. Recently, the development and characterization of a first-in-class novel azole analog [1-(4-(4-(((2R,4S)-2-(2,4-difluorophenyl)-2-methyl-1,3-dioxolan-4-yl)methoxy)phenyl)piperazin-1-yl)ethanone (FLB-12)] that antagonizes the activated state of PXR has been published (Venkatesh et al., 2011). This analog has limited effects on other related nuclear receptors LXR, FXR, estrogen receptor α, PPARγ, and mouse CAR. FLB-12 was demonstrated to abrogate endogenous PXR activation in vitro and in vivo and was less toxic to liver cells in vivo compared to ketoconazole. Interestingly, FLB-12 significantly abrogates PXR-mediated resistance to 7-ethyl-10-hydroxycamptothecin (SN-38) in colon cancer cells in vitro. These drugs will not only serve as valuable chemical tools for probing PXR action but will also be important adjuncts for novel targeted approaches against cancer drug resistance.

Thus, the concept that down-regulating PXR can sensitize cancer cells to chemotherapeutic agents has been proposed and investigated in several studies. In the prostate cancer cell line PC-3, treatment with the PXR agonist SR12813 activates PXR and increases both the expression of MDR1 and the resistance of PC-3 cells to the anticancer drugs paclitaxel and vinblastine. Inversely, the targeted knock-down of PXR by using short hairpin RNA (shRNA) enhanced the sensitivity of PC-3 to paclitaxel and vinblastine, suggesting that the effectiveness of anticancer drugs can be enhanced in PXR-positive cancers by decreasing the expression of PXR. Down-regulation of PXR by small interfering RNA (siRNA) in the endometrial cancer cell line HEC-1 also decreased the expression of MDR1 and sensitized cells to anticancer agent and PXR agonist paclitaxel and cisplatin (Masuyama et al., 2003; Masuyama et al., 2007). Other reports suggest that down-regulation of PXR may contribute to apoptotic and drug sensitivity in cancer cells (Gong et al., 2006; Masuyama et al., 2007). Finally, expression of PXR in human colorectal cancer cells led to irinotecan chemoresistance through enhancement of its glucuronidation catalyzed by UGT1A1. The opposite effect was obtained with pharmacological inactivation of PXR or shRNA-mediated PXR down-regulation, confirming the direct involvement of PXR in irinotecan chemoresistance (Raynal et al., 2010). Altogether, these studies demonstrate that PXR represents a potential therapeutic target for clinical applications relevant drug resistance.

Although the properties of CAR and its agonists in xenobiotic metabolism have been extensively studied, its anticancer property was not known until very recently. Indeed, a recent study showed that CAR is a positive regulator of MDR1 (Pgp), MRP2 and BCRP expression in rat and mouse brain capillaries (Wang, B. et al., 2010). Moreover, another study demonstrated that CITCO inhibits the growth and expansion of brain tumor cancer stem cells by inducing cell cycle arrest and apoptosis in vitro (Chakraborty et al., 2011). Although the CAR-mediated antineoplastic effect is not known, these results support the
use of CAR agonists as a new therapy to target brain tumor cancer stem cells for the treatment of glioma.

### 4.1.2 HIF-1 and its target genes

The adaptive cellular response of cancer cells to hypoxia offers new pharmacological targets, including the central regulator of molecular and cellular response to hypoxia HIF-1 as well as some of its target genes, particularly the VEGF and the carbonic CA9 (see Table 1). Validation of HIF-1 as a therapeutic target has been based on studies using genetic manipulation. When HIF-1α expression is increased in human cancer cells, angiogenesis capacity and metastasis spread are observed. Conversely, inhibition of the HIF-1α expression reverses those effects (Semenza, 2007). Accordingly, injection of tumor cells overexpressing HIF-1α into immunodeficient mice has demonstrated the capacity of HIF-1 to promote tumorigenesis (Maxwell et al., 1997). A growing number of novel anticancer agents have been shown to inhibit HIF-1 through a variety of molecular mechanisms. One of these promising molecules, the YC-1 ((3-(5′-Hydroxymethyl-2′-furyl)-1-benzylindazole), decreases the levels of HIF-1α protein through inhibition of the PI3K/AKT/mTor pathway (Sun et al., 2007). It has been shown that inhibition of HIF-1α activity in tumors from YC-1-treated mice is associated with blocked angiogenesis and an inhibition of tumor growth (Yeo et al., 2003).

<table>
<thead>
<tr>
<th>Target</th>
<th>Agent</th>
<th>Mechanism of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypoxia</td>
<td>Mitomycin C</td>
<td>DNA damages</td>
</tr>
<tr>
<td></td>
<td>Banoxantrone (AQ4N)</td>
<td>DNA damages and topoisomerase II inhibitor</td>
</tr>
<tr>
<td></td>
<td>Tirapazamine (TPZ)</td>
<td>DNA damages</td>
</tr>
<tr>
<td>HIF-1 pathway</td>
<td>YC-1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>PI3K/AKT/mTor inhibitor</td>
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<tr>
<td></td>
<td>Tanespimycin (17-AAG)</td>
<td>HSP90 inhibitor</td>
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<td></td>
<td>PX-12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Thioredoxin inhibitor</td>
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<td></td>
<td>Topotecan</td>
<td>Topoisomerase I inhibitor</td>
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<tr>
<th>HIF-1 target genes</th>
<th>Agent</th>
<th>Mechanism of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA9</td>
<td>CAI17</td>
<td>CA9-specific small molecule inhibitor</td>
</tr>
<tr>
<td>VEGF</td>
<td>Sorafenib</td>
<td>Tyrosine kinase inhibitor</td>
</tr>
<tr>
<td></td>
<td>Bevacizumab</td>
<td>Anti-VEGF antibody</td>
</tr>
<tr>
<td>GLUT1</td>
<td>Fasentin</td>
<td>Interacts with GLUT1 transporter and block glucose uptake</td>
</tr>
</tbody>
</table>

Table 1. Examples of pharmacological approaches to target hypoxic cancer cells.  
<sup>a</sup> 3-(5′-Hydroxymethyl-2′-furyl)-1-benzylindazole; <sup>b</sup> 1-methylpropyl 2-imidazolyl disulfide.

Angiogenesis has been described as one of the hallmarks of cancer, playing an essential role in tumor growth, invasion, and metastasis. For this reason, inhibition of angiogenesis has become a major challenge in the development of new anticancer agents, particularly in targeting the VEGF pathway. Sorafenib, a multtargeted inhibitor of tyrosine kinase, inhibits the receptor tyrosine kinase VEGFR2 and PDGFR and the Ras/Raf pathway.
Anticancer Drug Metabolism: Chemotherapy Resistance and New Therapeutic Approaches (Keating and Santoro, 2009). Currently, this anticancer molecule demonstrated encouraging result for palliative therapy and can prolong the overall survival for patients with advanced hepatocellular carcinoma (Cheng et al., 2009). Moreover, anti-angiogenic therapy seems efficient to improve survival from patients with hepatocellular carcinoma and the anti-VEGF monoclonal antibody bevacizumab has shown promising results (Llovet and Bruix, 2008).

Increased expression of CA9 has been found in many cancers and has been associated to an unfavorable prognosis (Kaluz et al., 2009; Pastorekova et al., 2008; Potter and Harris, 2004). Silencing both CA9 and CA12 resulted in marked inhibition of the growth of LS174 human colon carcinoma cell xenograft tumors (Chiche et al., 2010). Therefore, CA9 seems to be a new candidate for the development of new anticancer strategy. Interestingly, novel CA9-specific small molecule inhibitors such as the sulfonamide-based CAIX inhibitor CAI17 resulted in significant inhibition of tumor growth and metastasis formation in both spontaneous and experimental models of metastasis.

4.2 Bioreductive agents

It has been suggested that hypoxic environment in tumor tissues could be used as an advantage to target cancer cells with prodrugs that are metabolized into toxic metabolites only in hypoxic areas (McKeown et al., 2007). These drugs, also named bioreductive agents, are divided into 4 groups: quinones, nitroaromatics or nitro-heterocyclic, aliphatic N-oxides and heteroaromatic N-oxides.

Mitomycin C that belongs to the quinone family is an alkylating antineoplastic agent and is frequently used for chemoembolization therapy. Bioreduction and activation of mitomycin C are facilitated upon a hypoxic environment. Indeed, electrons gain (reduction) of mitomycin products a semiquinone radical anion, which forms a covalent interaction with DNA. In the presence of oxygen, this radical anion is quickly degraded, thus giving the selectivity of hypoxia for generation of cytotoxic species (Kennedy et al., 1980).

AQ4N or banoxantrone, is part of the aliphatic N-oxides. AQ4N is reduced into AQ4 under hypoxic condition. AQ4 exerts its cytotoxic activity by binding DNA and acting as an inhibitor of topoisomerase II. Used in combination with other anticancer agents, it has anti-proliferative effects on tumor cells (Patterson and McKeown, 2000).

4.3 Activation of prodrugs by glutathione transferases

As previously mentioned, a feature of cancer cell is to overexpress certain drug-metabolizing enzymes and transporters. Pathways involving such proteins that are aberrantly expressed in cancer cells are preferentially targeted for drug intervention. For example, the enhanced expression of GSTP1 in several tumors makes this protein a promising target for prodrug therapy. In order to take advantage of GSTP1 overexpression in cancer cells, two strategies have been performed. The first one consists in designing and developing inhibitors of GSTP1. Initially, this strategy was developed in order to decrease the metabolism of several active anticancer drugs known to be inactivated by GST. Furthermore, in 1999, evidence for a direct interaction of mouse GST pi with JNK was demonstrated (Adler et al., 1999). Their work showed that, under a monomeric state, GST pi
acts as a direct JNK inhibitor in non-stressed cells by forming a complex with JNK and c-Jun. Oxidative stress (UV, H$_2$O$_2$...) induces the dimerization of GST pi and activation of c-jun through its phosphorylation on Ser-63 and Ser-73 residues. Subsequently, several other studies have corroborated this model in other cell lines (Bernardini et al., 2000; Castro-Caldas et al., 2009) and have shown that overexpression of GSTP1 in several tumor tissues lead to an inhibition of apoptosis pathways. Thus, inhibitor of GSTP1 triggering the disruption of this interaction could induce apoptosis in the cancer cell. TLK199 is one of them (Raza et al., 2009). The second strategy consists in designing prodrug activated by this enzyme in order to target specifically the tumor cells overexpressing GSTP1. Thus, novel alkylating agents have been synthetized (Lyttle et al., 1994; Satyam et al., 1996). Cleavage of these molecules by GSTP1 lead to the release of two metabolites: an inactive GSH conjugate and a phosphorodiamide compound. The phosphorodiamide spontaneously gives an alkylating moiety (a nitrogen mustard alkylating agent) which is responsible for the cytotoxicity. Among all the products synthetized, one has been actively studied and is tested in phase 2 and 3 studies (Kavanagh et al., 2010; Vergote et al., 2009). Initially named TER286, then TLK286, it is now designate with the International Nonproprietary Name (INN) canfosfamide (Telcita®). Several in vitro and in vivo studies, either on cell lines or on xenograft models, have linked the cytotoxicity of this molecule with the high level expression of GSTP1 and the formation of the alkylating moiety (Izbicka et al., 1997; Morgan et al., 1998; Rosario et al., 2000). Furthermore, Townsend et al. (Townsend et al., 2002) have demonstrated that canfosfamide is able to inhibit an enzyme involved in double strand break DNA repair, the DNA-dependent protein-kinase (DNA-PK). Interestingly, up-regulation of this DNA-PK leads to a resistance of adriamycin and cisplatin, suggesting that canfosfamide could be used in combination with these drugs. Several phase I studies have been realized in order to determine the safety and the pharmacokinetic of canfosfamide in human. These tests have been performed in advanced refractory solid cancers and have shown that canfosfamide is well tolerated with mild or moderate adverse effects such as nausea, vomiting, fatigue and anemia (grade 1 or 2) (Rosen et al., 2003; Rosen et al., 2004). Furthermore canfosfamide seems to be active in a large range of cancer including advanced non-small cell lung tumor (Sequist et al., 2009). Phase 2 and 3 clinical studies have also been done on resistant epithelial ovarian cancer (Kavanagh et al., 2010; Vergote et al., 2009).

Another family of compounds is under development. These compounds own an O$_2$-aryl diazeniumdiolate structure and are also metabolized by GSTP1 in a non-stable metabolite owning a Meisenheimer complex intermediate, which gives a GSH metabolite (PABA-GSH) and nitrogen monoxide (NO). Several of them have been designed (Andrei et al., 2008; Chakrapani et al., 2008; Saavedra et al., 2006) but the most specific and the most studied is the PABA/NO (O2-[2,4-dinitro-5-(p-methylaminobenzoato)] 1-(N, N-dimethylamino)diazen-1-i um-1,2-diolate) (Ji et al., 2008). Antiproliferative proprieties have been observed in several cell lines, including the mouse skin fibroblast NIH3T3 (Findlay et al., 2004), the human promyelocytic leukemia HL60 (Hutchens et al., 2010), the human leukemia U-937, the non-small-cell lung cancer H441, the colon cancer (HCT-116, HCT-15 and HT-29), the ovarian cancer OVCAR-3 (Andrei et al., 2008) and the U87 gliomas cell lines (Kogias et al., 2011). Antitumor activity was also demonstrated in an A2780 human ovarian cancer xenograft model in female SCID mice (Findlay et al., 2004). Mechanisms of cytotoxicity of PABA/NO involve several pathways which are due to the NO production and the nitrosylation and S-
glutathionylation of some proteins. Townsend et al. (2005) have shown that PABA/NO is able to induce S-glutathionylation of several proteins, including the protein disulfide isomerase (PDI) (Townsend et al., 2005). Glutathionylation of PDI triggers a decrease of the folding protein capacity response, leading to cytotoxic effects. Activation of the apoptosis pathway through activation of JNK and p38 has also been observed (Townsend et al., 2005). Furthermore, GSH metabolite of PABA/NO is also able to inhibit sarco/endoplasmic reticulum calcium ATPases iso-enzymes, leading to an intracellular Ca\(^{2+}\) increase, triggering activation of the calmodulin pathway and thus increasing production of NO by endothelial Nitric Oxide Synthase (Manevich et al., 2010).

5. Conclusion

During the last years, several mechanisms involved in resistance phenomena have been elucidated and showed that, in many cases, drug-metabolizing enzymes and drug transporters are key factors in the failure of cancer therapies. In some cases, these discoveries led to useful strategies to identify “sensitive” tumors and direct clinical decisions for the choice of therapy. Furthermore, the molecular classification of several tumor types based on genome-wide investigations and identification of patient subclasses according to drug responsiveness should help to propose a more personalized medicine and to overcome anticancer drug resistance. Another promising field of investigation is to take advantage of cancer cell specificity in order to develop new tumor-targeted approaches that afford tumor specificity and limited toxicity.

6. References


Baskin-Bey, E.S., Huang, W., Ishimura, N., Isomoto, H., Bronk, S.F., Braley, K., Craig, R.W., Moore, D.D., and Gores, G.J. (2006). Constitutive androstane receptor (CAR) ligand,


Shibata, T., Kokubu, A., Gotoh, M., Ojima, H., Ohta, T., Yamamoto, M., and Hirohashi, S. (2008a). Genetic alteration of Keap1 confers constitutive Nrf2 activation and


In order to avoid late-stage drug failure due to factors such as undesirable metabolic instability, toxic metabolites, drug-drug interactions, and polymorphic metabolism, an enormous amount of effort has been expended by both the pharmaceutical industry and academia towards developing more powerful techniques and screening assays to identify the metabolic profiles and enzymes involved in drug metabolism. This book presents some in-depth reviews of selected topics in drug metabolism. Among the key topics covered are: the interplay between drug transport and metabolism in oral bioavailability; the influence of genetic and epigenetic factors on drug metabolism; impact of disease on transport and metabolism; and the use of novel microdosing techniques and novel LC/MS and genomic technologies to predict the metabolic parameters and profiles of potential new drug candidates.

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