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Recent Advances on the Molecular Mechanisms Involved in the Drug Resistance of Cancer Cells and Novel Targeting Therapies

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Abstract

This review summarizes the recent knowledge obtained on the molecular mechanisms involved in the intrinsic and acquired resistance of cancer cells to current cancer therapies. We describe the cascades that are often altered in cancer cells during cancer progression that may contribute in a crucial manner to drug resistance and disease relapse. The emphasis is on the implication of ATP-binding cassette (ABC) multidrug efflux transporters in drug disposition and antiapoptotic factors, including epidermal growth factor receptor cascades and deregulated enzymes in ceramide metabolic pathways. The altered expression and activity of these signaling elements may have a critical role in the resistance of cancer cells to cytotoxic effects induced by diverse chemotherapeutic drugs and cancer recurrence. Of therapeutic interest, new strategies for reversing the multidrug resistance and developing more effective clinical treatments against the highly aggressive, metastatic, and recurrent cancers, based on the molecular targeting of the cancer progenitor cells and their further differentiated progeny, are also described.

Important advances in the development of novel early diagnostic and prognostic methods and therapeutic treatments of cancers using surgical tumor resection, hormonal therapies, radiotherapy, or adjuvant chemotherapy, alone or in combination, have been achieved in past years.[1–13] This has led to a substantial increase in the cure rate for patients diagnosed in the early stages of localized cancers. For patients diagnosed in the late stages of locally invasive and metastatic cancers, the systemic chemotherapeutic regimens represent one of the principal clinical options. In general, the current chemotherapeutic treatments may contribute to enhancing the time to disease progression, overall survival, and quality of life for patients with advanced and aggressive disease states. Unfortunately, current chemotherapeutic treatments for advanced cancers often result in disease relapse and ultimately lead to the death of the patients.[2–6,9,11,12,14–19] The development of resistance by cancer cells to hormonal therapies, radiotherapy, and chemotherapeutic drugs, which usually occurs during cancer progression and after long-term treatment, still represents a major challenge in the clinical cure of advanced and metastatic cancer forms. Therefore, this underlines the critical importance of establishing

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molecular mechanisms involved in the drug disposition and resistance or multidrug resistance (MDR) of cancer cells for improving current therapies against aggressive cancers in the clinics. Numerous works have indicated that the alterations in diverse signaling elements may contribute to high levels of resistance to one or some chemotherapeutic drugs and radiation. [20–26] Among them are the elevated expression and activity of ATP-binding cassette (ABC) multidrug efflux pumps, DNA repair enzymes, and growth factor signaling elements, including epidermal growth factor receptor (EGFR), hedgehog, and Wnt/*β*-catenin, as well as alterations in ceramide metabolism enzymes and changes in expression levels of drug targets or the inactivation of drugs.

Recent lines of evidence have also revealed that the accumulation of genetic or epigenetic alterations, including mutations in adult stem cells or early progeny during their lifespan, may lead to their malignant transformation into leukemic or tumorigenic cancer progenitor cells, also designated as cancer stem cells or cancer-initiating cells.[3,12,13,21,27–39] The leukemic or tumorigenic cancer progenitor cells may acquire a more malignant phenotype during cancer progression and give rise to further differentiated cancer cell sub-populations constituting the primary and secondary neoplasms. In support of this, a population of cancer progenitor cells representing a very small fraction of total cancer cells and expressing the specific stem cell-like markers, including CD133, CD44, Sca-1, Oct-3/4, c-KIT, and/or ABC multidrug efflux transporters, has been identified in numerous cancers. Among them are leukemias and diverse human solid tumor types, including skin, lung, brain, breast, ovarian, prostate, pancreatic, gastrointestinal, and colorectal cancers.[12,13,15,27,29,32,39–48] It has been observed that a small number of these cancer progenitor cells isolated from the patients' malignant tissue specimens can initiate and drive leukemia or tumor progression in animal models *in vivo*, whereas the bulk mass of further differentiated cancer cells was non-leukemic, non-tumorigenic, or only formed, tumors when implanted in very high numbers. [27,29,32,41,42,44,45,47,48] Hence, the differences between the functional properties of leukemic or tumorigenic cancer progenitor cells versus their further differentiated progeny, emphasize the importance of considering the intrinsic properties of these cancer-initiating cells to overcome resistance to current cancer therapies. Indeed, the leukemic or tumorigenic cancer progenitor cells, similar to their normal counterpart, the adult stem cells, generally display an active DNA repair and high-expression levels of some ABC multidrug efflux pumps, antiapoptotic factors, and inhibitors of apoptosis (Figure 1).[13,15,21,24,49,50] The intrinsic properties of cancer progenitor cells may provide them a greater resistance to current clinical therapies than that observed for their further differentiated progeny. Furthermore, the reactivation of diverse developmental signaling pathways involved in stem cell self-renewal, including EGFR, hedgehog, and Wnt/*β*-catenin, which frequently occurs in cancer progenitor cells during cancer initiation and progression, may contribute to their sustained growth, survival, drug resistance, and disease relapse (Figure 1).[12,13,25,26,30,31,39,51–53] The host cells, including the tumor fibroblasts, infiltrating macrophage, and endothelial cells, may also influence the behavior of cancer progenitor cells during tumor progression.[12,39,54] Thus, the persistence of leukemic or tumorigenic cancer progenitor cells at the primary or metastatic sites after the current clinical treatments may be responsible, at least in part, for the recurrence of the most aggressive cancers. In this matter, we report the molecular mechanisms often associated with the resistance of cancer cells to current cancer therapies and disease relapse. We also describe new therapeutic strategies based on molecular targeting for deregulated signaling elements involved in the survival and resistance of cancer cells, particularly of cancer stem/progenitor cells.

CANCER THERAPIES AGAINST LOCALLY ADVANCED AND METASTATIC CANCERS

Current clinical cancer therapies

Significant therapeutic advances have led to the cure of patients diagnosed with diverse types of localized cancers in the clinics.[1–13] Nevertheless, most patients who undergo potentially curative tumor resection, hormonal therapy, and radiotherapy for advanced and metastatic cancers subsequently relapse because of the persistence of cancer cells in primary tumors and micrometastases.[3,11–13,15,19,34,39,43,55–58] Therefore, systemic chemotherapy may represent another option to eradicate the cancer cells at the primary and distant metastatic sites. Several preclinical and clinical trials have been performed to increase the efficacy of current chemotherapeutic regimens used for the treatment of patients with locally advanced and metastatic cancers in the clinics. Although there have been important advances, the current chemotherapeutic regimens remain ineffective against the most advanced and metastatic cancers. This is partly due to dose-limiting toxicity that restricts their use at high doses, and to the development of diverse drug resistance and MDR mechanisms by cancer cells. It has been observed that the combinations of chemotherapeutic drugs, which target distinct oncogenic signaling elements, are generally more effective than individual drugs.[2,3,12,13,59] The combination of low doses of drugs may decrease certain side effects associated with the use of high concentrations of single drugs. Of particular interest, the combined use of high-dose chemotherapy, high-dose sequential chemotherapy, or highintensity ionizing radiation with subsequent autologous or allogenic stem cell transplantation support may also constitute alternative effective strategies. These therapeutic approaches are particularly considered among the principal options in the clinics for patients with high-risk or relapsed/refractory cancers. These treatment types can be used to treat leukemias, multiple myeloma, and Hodgkin's and non-Hodgkin's lymphomas as well as advanced and metastatic solid tumors such as sarcoma, neuroblastoma, medulloblastoma, retinoblastoma, and lung, breast, kidney, ovarian, and colorectal cancers.[13,60–72] For instance, the results from two prospective phase II trials have revealed that high-dose sequential chemotherapy followed by autologous peripheral blood stem cell transplantation resulted in an estimated 12-year overall, disease- and event-free survival of 34, 55, and 30%, respectively, in 46 patients diagnosed with advanced stage peripheral T-cell lymphomas.[65] It has also been noted that this treatment type, given a higher rate of long-term complete remission in patients with anaplastic lymphoma-kinase-positive anaplastic large-cell lymphoma and complete remission before autografting, may represent an important factor indicative of a better prognosis of patients.[65] Thus, the choice of the therapeutic treatments, including the regimen option, drug, doses, and administration sequence of combined drugs should be optimized and adapted to each patient in a particular clinical setting. The clinical genetic analyses, including the oncogenic gene expression profiling of patients, may notably improve the diagnostic and prognostic tests in certain cancer cases. It may also help to establish the most appropriate types of therapeutic intervention to counteract the recurrence of the disease.

Pharmacogenetic analyses

Numerous pharmacogenetic investigations have revealed that the interindividual differences in genetic polymorphisms and mutations may influence the risk for developing certain cancer types and the patient's response to a particular therapeutic approach. [73–77] Particularly, the genetic polymorphisms or mutations in the drug targets, the transporters or metabolizing enzymes involved in drug disposition and DNA repair enzymes, may contribute to the variability in drug pharmacokinetic and pharmacodynamic processes and also drug response and observed toxicity.[73–75,77,78] For instance, certain polymorphisms in human multidrug transporter gene *ABCB1* encoding P-glycoprotein (P-gp) or DNA repair enzymes such as

excision-repair cross-complementing group 1 protein might be associated with the chemotherapy response observed in lung cancer patients.[79,80] Similarly, the occurrence of EGFR gene polymorphisms or mutations may also influence the response to therapeutic cancer treatment and outcome of patients.[74,76]

Some studies have also revealed that the risk of disease relapse, which is generally associated with the presence of micrometastases at distant sites, might be predicted by the specific gene expression profile in the primary neoplasm, whose signature may be indicative of the rate of recurrence and the overall survival of patients.[14,81–85] Thus, pharmacogenetic analyses in patients' cancer tissue samples by methods of genotyping, such as traditional DNA sequencing and microarray technology, could aid in determining the potent resistance or response of patients to a particular type of cancer therapy. The gene expression profiling of diverse human cancer tissue samples, made before or after adjuvant chemotherapy, has provided important information on the molecular signatures that may aid in predicting the potent chemoresistant phenotype and the prognosis of patients.[86–89] For instance, the microarray analyses of gene expression patterns in leukemic blasts from 360 pediatric patients with acute lymphoblastic leukemia revealed that the gene expression profiles may help to identify the patients at high risk for failing therapeutic treatments.[88] The DNA microarray screening of the expression of approximately 21,000 genes in paired tumor samples, taken before and after chemotherapeutic treatment from six patients with predominantly advanced stage, high-grade epithelial ovarian cancers, has revealed that the intrinsic and acquired chemoresistant phenotypes of post-chemotherapeutic tumors, relative to primary tumors, may be attributed to the combined action of different factors.[87] These factors may be implicated in regulatory mechanisms of cell proliferation, tumor progression, and chemoresistance.[87] In addition, the cytogenetic analyses of 23 cancer cell lines, made resistant to either camptothecin, cisplatin, etoposide (VP-16), doxorubicin, or 1-β-D-arabinofuranosylcytosine, have also revealed that the genomic alterations in the copy numbers of certain genes may be a characteristic related to the acquisition of drug resistance mechanisms in cancer cell lines.[90] More specifically, the amplification of several ABC multidrug efflux transporter genes, such as multidrug resistance 1 (MDR1), multidrug resistance-associated protein 1 (MRP1), and antiapoptotic factor Bcl-2, or a decrease in the copy numbers of genes encoding deoxycytidine kinase, DNA topoisomerase I, and DNA topoisomerase II-α were observed in certain drug resistance cancer cells examined, as compared to drug-sensitive parental cancer cells.[90] A recent study has also indicated that an increase of MRP1 expression in a subset of patients with breast cancer may be associated with the resistance to standard adjuvant cyclophosphamide, methotrexate, fluorouracil chemotherapy, but it does not alter the response to tamoxifen and goserelin.[91] This suggests that the analyses of the MRP1 expression could aid in determining whether the adjuvant endocrine treatment could be beneficial to estrogen receptor alpha (ERα)-positive patients. Thus, on the basis of these observations, it appears that the clinical pharmacogenetic analyses of biomarkers of sensitivity of cancer cells to specific drug treatments could be beneficial in certain cancer cases. For instance, the analyses of EGFR expression levels and the mutational status in biopsy material could be used to select patients who would benefit from a clinical treatment with the selective EGFR inhibitors such as gefitinib. Hence, the identification and molecular targeting of signaling elements involved in self-renewal, invasion, and the drug resistance of cancer cells may constitute a novel promising approach for improving the current chemotherapeutic treatments of patients with aggressive and metastatic cancers in the clinics.[15,92] We describe here the molecular mechanisms that may contribute to the intrinsic and acquired resistance phenotypes of cancer cells, and that may influence their sensitivity to conventional cancer therapies and disease relapse.

Drug resistance mechanisms of cancer cells and targeting therapies

A major problem in the clinical treatment of advanced and metastatic cancer forms is the intrinsic or acquisition of a resistant phenotype by cancer cells to current therapeutic treatments.[21] Particularly, the acquisition of the MDR phenotype by cancer cells, which may occur after exposition to one specific drug, and may result in the cross-resistance to other structurally and functionally unrelated anticancer drugs.[93,94] Drug resistance mechanisms, including the MDR phenomenon, may be associated with alterations in the expression and activity of a complex framework of signaling elements that actively protect the cancer cells from the cytotoxic effects induced by xenobiotics, including chemotherapeutic drugs (Figure 1). Therefore, the assessment of the multiple factors related to the chemotherapeutic drug resistance is necessary for the development of new drugs and therapeutic regimens for a more effective treatment of aggressive and recurrent cancers.

Many metastatic cancer cell lines established from skin, brain, non-small-cell lung (NSCLC), prostate, breast, ovarian, pancreatic, and colorectal cancers show altered expression and activation of diverse signaling cascades. Among them, there are enhanced activation of diverse developmental signaling pathways, including EGFR, hedgehog, and Wnt/β-catenin as well as certain signaling elements, such as PI_3K (phosphatidyl inositol 3′-kinase)/Akt, nuclear factor-κB, Bcl-2, cyclooxygenase-2 (COX-2), survivin, snail, slug, and twist. These pathways may contribute to their uncontrolled growth, survival, invasion, and resistance to conventional therapies (Figure 1).[12,13,22,23,25,26,30,31,39,51,52,95] The establishment of cancer cell lines showing high levels of drug resistance has allowed researchers to identify certain deregulated gene products that may contribute to the acquired resistance of cancer cells to antineoplastic drugs. Among them are the elevated expression and activity of ABC multidrug efflux pumps, DNA repair enzymes, and growth factor signaling elements, including EGFR, hedgehog, and Wnt/β-catenin. Moreover, the alterations in apoptotic signaling elements such as ceramide metabolism enzymes and changes in expression levels of drug targets and the inactivation of drugs may also provide cancer cells with a high resistance to one or some chemotherapeutic drugs and radiation.[20–26] Therefore, specific inhibitory agents that target signaling pathway elements involved in cancer progression, invasion, and resistance to conventional cancer treatments are of therapeutic interest. These agents could be used in novel clinical therapeutic regimens and in improving current treatments against aggressive, metastatic, and recurrent cancers (Table 2).[11,25,96] Regardless, we report, in a more detailed manner the implication of ABC multidrug efflux transporters in the drug disposition, EGFR-dependent signaling pathways, and the enzymes involved in the ceramide metabolism as well as the novel therapeutic agents developed for their molecular targeting. Of particular therapeutic interest, we present an overview of the recent knowledge obtained on specific alterations that may contribute to the intrinsic and acquired resistance and decreased response of leukemic or tumorigenic cancer stem/progenitor cells to antineoplastic drugs. We also discuss the critical importance of considering their molecular targeting for improving the current clinical cancer therapies.

Targeting of ABC multidrug efflux pumps

The different members of the ABC multidrug efflux transporter superfamily, which are expressed in a variety of tissues/organs, are constituted of either one or two transmembrane spanning domains involved in drug binding and one or two cytoplasmic nucleotide (ATP)-binding domains (Figure 1).[17,24,97–103] One of the physiological roles of these cellular transporters is to pump out diverse endogenous substrates at the expense of ATP hydrolysis. Hence, they may protect the normal tissues from the cytotoxic effects of xenobiotics, including the drugs. Each ABC transporter family, however, possesses the specific structural and functional properties and is able to transport only a specific range of several classes of anticancer drugs (Table 1).[17,24,97–103] The best characterized ABC transported superfamily

members include MDR1 gene product P-gp, MRPs, such as MRP1 and MRP2, and breast cancer resistance protein (BCRP)/ABCG2, also designated as mitoxantrone resistance (MXR) and placenta-specific ATP-binding cassette protein (ABCP) (Figure 1).[20,24,97–104] A substantial contribution of the multidrug transporters, P-gp and MRP1, to basal drug resistance has been shown by using engineered mouse fibroblast cell lines, in which the genes encoding for these proteins were inactivated.[20] The cell lines lacking functional P-gp were markedly more sensitive to paclitaxel (PTX, 16-fold), anthracyclines (fourfold), and *Vinca* alkaloids (threefold) than the wild-type cell lines (Table 1). The fibroblast cell lines lacking both P-gp and MRP1 proteins were also highly sensitive to a large range of drugs, including epipodophyllotoxins (four- to sevenfold), anthracyclines (six- to sevenfold), camptothecin (threefold), and *Vinca* alkaloids, especially vincristine (28-fold), relative to parental cell lines.[20] This suggests that a small change in the expression levels of these drug transporters may have a major repercussion on the sensitivity of cancer cells to chemotherapeutic drugs. In addition, the characterization of diverse cancer cell lines showing an acquired resistance to one or several anticancer drugs has allowed researchers to identify certain ABC transporters that may be activated in malignant cells and contribute to drug resistance. For instance, the enhanced expression of P-gp has been associated with the acquired decreased sensitivity of doxorubicin-resistant DU145 and PC3 prostatic cancer cell sublines to various anticarcinogenic drugs. Among them are etoposide, camptothecin, vinblastine, vincristine, fluorodeoxyuridine, doxorubicin, amsacrine, and melphalan (Table 1).[105] The implication of the ABC transporter P-gp in drug resistance was further supported by the observation that the treatment of doxorubicin-resistant DU145 and PC3 cells with a P-gp inhibitor, verapamil, restored their sensitivity to doxorubicin.[105] An immunohistochemical analysis of BCRP/ABCG2 expression levels in 150 untreated tumors has also revealed that this transporter is often expressed in several cancer types, specifically, in the carcinomas of the gastrointestinal tract.[106] Moreover, the enhanced expression of the gene and protein levels of BCRP/ABCG2 multidrug efflux transporter has been associated with the enhanced resistance to diverse chemotherapeutic drugs, including mitoxantrone, irinotecan, SN-38, topotecan, and anthracycline (Table 1).[24,102] For instance, the enhanced expression of BCRP/ABCG2 protein in SN-38-resistant NSCLC cell line NCI-H23 resulted in a decreased sensitivity to SN-38. This was also accompanied by cross-resistance to other anticancer drugs such as topotecan, etoposide, doxorubicin, and mitoxantrone, relative to the parental cell line.[107] More specifically, the cellular accumulation of topotecan was decreased in SN-38-resistant NCI-H23 cells compared with that in the parental SN-38-sensitive NCI-H23 cells, and the treatment of SN-38-resistant NCI-H23 with an inhibitor of BCRP/ABCG2 transporter reversed this effect.[107] A high level of ABCA2 transporter, which is localized in the endolysosomal compartment in ovarian and small-cell lung cancer cell lines, has also been related to resistance to estramustine and mitoxantrone, respectively.[100,108,109] This suggests the possible implication of ABCA2 transporter in lysosomal detoxification and MDR phenomenon in these cancer cell lines. Moreover, the results of microarray and real-time polymerase chain reaction analyses have also indicated that the ABCA2 and ABCA3 transporters are overexpressed in pediatric T-cell acute lymphoblastic leukemia and acute myeloid leukemia compared with healthy bone marrow. The enhanced expression of these transporters may contribute to resistance to methotrexate, vinblastine, or doxorubicin.[110,111]

ABC transporter protein inhibitors—As ABC transporter superfamily members provide a critical function in the acquisition of an MDR phenotype of cancer cells, much effort has been made to overcome MDR mediated through these drug efflux pumps. Strategies developed to reverse MDR include the use of pharmacological agents that can interact with one or several ABC transporters, thereby inhibiting their functions, monoclonal antibody directed against a specific ABC transporter, and antisense oligonucleotide or oligodeoxynucleotide (Figure 2 and Table 2).[17,24,103,112–118] An alternative approach also considered using agents that can inhibit

the signaling cascade elements responsible for the enhanced expression of ABC transporters in resistant cancer cells.[114] Certain pharmacological agents have been identified and observed to inhibit the transport activity of P-gp, MRPs, and BCRP/ABCG2 efflux pumps, increase the intracellular drug accumulation, and reverse MDR *in vitro* and *in vivo*, and in certain cases have reached clinical trials. Unfortunately, few successful treatments have been developed based on their use in combination with other conventional chemotherapeutic drugs. The lack of clinical efficacy of most of the ABC transporter modulatory agents tested appears to be due, in part, to their low oral bioavailability, high cellular extrusion and metabolism, additional cellular targets, and cellular systemic toxicity at effective doses.[101,119,120] For instance, P-gp transporter protein inhibitors such as verapamil and cyclosporin A, which are used in other clinical therapeutic applications, as well as their analogues, dexverapamil and valspodar (PSC-833), have been observed to reverse the MDR mediated via the P-gp transporter in a variety of cancer cells *in vivo* and *in vitro*.[21,121] However, the results of phase III trials have indicated that these agent types show a less convincing response rate in clinical settings.[21, 121] A recent study has revealed that the combined application of different classes of P-gp modulators at low concentrations could represent a more promising approach for improving their efficacy and decreasing the toxicity associated with the use of high concentrations of these individual agents *in vivo*. For instance, it has been reported that the conformation-sensitive UIC2 monoclonal antibody directed against membrane P-gp protein partially inhibited the P-gp-mediated substrate transport when used alone (**Figure 2**). Interestingly, the simultaneous application of UIC2 with other modulators, such as cyclosporin A or valspodar, followed by their removal, resulted in nearly 100% inhibition of P-gp drug efflux pump activity.[122] This inhibitory effect was also accompanied by a complete restoration of intracellular accumulation of various P-gp substrates.[122] Further ongoing investigations also include the novel specific ABC transporter inhibitory agents acting on both P-pg and MRP1 drug efflux pumps, such as quinoline derivative dofequidar fumarate (MS-209) and biricodar (VX-710).[120,121,123] Among them, clinical trials with MS-209, a quinolone-derived sphingomyelin synthase inhibitor, have been performed for the treatment of drug-resistant cancers, including breast cancer and NSCLCs.[120,123,124] The results from a phase I trial revealed that the combined use of MS-209 with docetaxel does not significantly affect the non-hematological and hematological toxicities and has little effect on pharmacokinetics of docetaxel in a group of patients with advanced and metastatic cancers.[125] Moreover, data from another recent clinical trial have also revealed that orally active MS-209 plus cyclophosphamide, doxorubicin, and fluorouracil (CAF) therapy was well tolerated and improved the progression-free survival in patients with advanced or recurrent breast cancer who had not received previous therapy in comparison with CAF treatment alone.[123] Additional results from long-term clinical trials should confirm the potent benefit of including this agent type in conventional chemotherapeutic regimens to reverse MDR and improve the overall survival of patients.

Several dietary flavonoids, such as genistein, silymarin, quercetin, biochanin A, daidzein, naringenin, and kaempferol, may also inhibit several ABC transporters, including P-gp-, MRP1-, and BCRP/ABCG2-mediated cellular drug efflux, thereby reversing the drug resistance of cancer cells.[126–128] It has also been reported that use of flavonoid cocktails constituted by an equimolar concentration of several flavonoid compounds resulted in additive inhibitory effects on the activity of ABC multidrug efflux pumps.[126] This suggests that these natural compounds may constitute the potential adjuvant therapeutic agents to restore the sensitivity of cancer cells to chemotherapeutic drugs. In this matter, it is noteworthy that the flavonoids presented in diets may interfere with treatments that use other ABC transporter inhibitory agents. Additionally, recent lines of evidence have also revealed that certain clinical anticarcinogenic agents, such as anti-estrogens and tyrosine kinase inhibitors (TKIs), may inhibit or completely reverse intrinsic and acquired resistance of cancer cells to distinct chemotherapeutic agents and thereby restore their sensitivity to the cytotoxic effects of drugs. For instance, anti-estrogens, toremifene, tamoxifen, and its derivatives have been observed to

increase the sensitivity of breast, lung, gastrointestinal, bladder, and colorectal cancer cell lines to cytotoxic effects induced by anthracyclines, *Vinca* alkaloids, and taxanes, at least in part, by overcoming P-gp transporter-mediated resistance in these cancer cells.[129,130] Particularly, the tamoxifen derivatives may interact with the BCRP/ABCG2 drug efflux pump and thereby enhance the cellular accumulation of topotecan in BCRP-transfected K562 cells (K562/BCRP) and reverse SN-38 and mitoxantrone resistance.[131] breakpoint cluster region protein (BCR)-Abelson kinase (ABL) TKI, imatinib mesylate (ST1571), and certain EGFR-TKIs, such as gefitinib and CI1033, as discussed below, have also been reported to act as substrates or inhibitors of functions of P-gp and BCRP/ABCG2 transporters.[24,132–141] Hence, these TKIs can reverse MDR mediated by drug efflux pumps in cancer cells *in vitro* and *in vivo*.[24,132–141] The interaction of TKIs with these ABC transporters expressed in normal tissues may also influence their pharmacokinetics and thereby contribute to resistance and toxicity associated with the use of these agents *in vivo*.[142,143]

Targeting of EGFR signaling pathway

The overexpression of EGFR (ErbB1) and its ligands EGF, transforming growth factor-β, heparin-binding EGF-like growth factor, and amphiregulin has been observed to occur during the progression of numerous human aggressive and metastatic cancers, including head and neck squamous cell carcinoma, NSCLC, brain, skin, prostate, breast, ovarian, and gastrointestinal cancers. This event has been associated with the disease relapse and resistance to current hormonal therapies, radiotherapy, and chemotherapies.[2,3,12,13,22,23,39,52,95,144–148] The sustained activation of EGFR may lead to the activation of diverse tumorigenic intracellular cascades (mitogen-activated protein kinases, PI_3K/Akt, nuclear factor-κB, and phospholipase C-γ) and the enhanced expression of multiple targeted gene products (Bcl-2, survivin, COX-2, vascular epidermal growth factor, urokinase plasminogen activator, and snail) (Figure 1). These cascades may contribute to uncontrolled growth, survival, and invasion of cancer cells. As the EGFR signaling pathway assumes a pivotal role in cancer progression and disease recurrence, many preclinical and clinical investigations have been carried out with the anti-EGFR or EGF antibody, antisense oligonucleotide, and TKIs (Figure 2 and Table 2).[2,3,12,13,39,96,146,149–153] Many studies have revealed the beneficial effect of combining these agent types for improving the cytotoxic effects induced by anti-hormonal agents, radiation, and other chemotherapeutic drugs. For instance, it has also been observed that gefitinib, erlotinib, or cetuximab (IMC-C225) may induce additive or synergistic growth inhibitory and apoptotic effects in combination with the anti-androgenic agents, such as bicalutamide, smoothened hedgehog signaling inhibitor (cyclopamine), and diverse chemotherapeutic drugs on diverse cancer cell lines *in vitro* and *in vivo*.[2,3,12,13,39,144,151–154] Among them are human lung, prostate, ovarian, breast, colorectal, pancreatic, head and neck, bladder, and epidermoid cancer cell lines. Furthermore, the results from a recent investigation have also revealed that gefitinib may inhibit the invasion and migration of tamoxifen-resistant MCF-7 breast cancer cells and restore the sensitivity of MCF-7a cells obtained after long-term estrogen deprivation to tamoxifen and anastrozole.[95,155] The gefitinib treatment also restored the sensitivity of hormone-independent and Bcl-2-overexpressing MDR MCF-7/ADR cells to PTX, docetaxel, and IDN S109 *in vitro*.[156] As the activation of insulin-like growth factor-1 receptor (IGF-1R) signaling may lead to the resistance of human breast, prostate, and NSCLC cells to EGFR-TKI such as gefitinib or erlotinib, the inclusion of specific IGF-1R inhibitor, such as anti-IGF-1R antibody or NVP-AEW541, may constitute a strategy to prevent or reverse the resistance of these cancer cell types to these agents (Figure 2 and Table 2).[157,158] Gefitinib can delay the emergence of resistance to both estrogen deprivation or anti-estrogen fulvestrant on the ErbB2/Her2/Neu-overexpressing ERα-positive MCF7/HER-2/neu-18 xenograft model *in vivo*.[159] However, the treatment with gefitinib and estrogen deprivation or fulvestrant subsequently led to tumor re-growth, which was accompanied by a loss of ERα, IGF-1R, and an increase of p-HER-2 (phosphorylated human epidermal growth factor receptor 2) and phosphorylated serine|

threonine protein kinase (pAKT|PKB) levels (Figure 2 and Table 2).[159] This suggests the potential benefit of also including the inhibitors of HER-2 and Akt for patients with ERα/HER-2-positive breast cancer subtypes (Figure 2 and Table 2).[159] Moreover, the EGFR inhibitor, PD153035, has also been observed to restore the sensitivity of human melanoma cells and ovarian cancer cells to betulinic acid and PTX, respectively, by inhibiting the drug-induced survivin expression.[160,161] Similarly, the inhibition of ErbB1/ErbB2 by using lapatinib (GW572016) also markedly reduced survivin expression and induced apoptosis in ErbB2-overexpressing breast cancer cells compared with trastuzumab and gefitinib alone.[162]

Several recent studies have indicated that gefitinib may inhibit certain MDR ABC transporter family members, including MDRI/ABCB1 gene product P-gp and BCRP/ABCG2 protein in diverse cancer cell types, overexpressing these drug efflux pumps.[135–141] Hence, gefitinib may reverse ABC transporter-mediated MDR and enhance the apparent bioavailability and efficacy of coadministrated drugs that are the substrates of these transporters *in vitro* and *in vivo*. For instance, gefitinib may reverse SN-38 resistance in BCRP-transduced human myelogenous leukemia K562 (K562/BCRP) or BCRP-transduced murine lymphocytic leukemia P388 (P388/BCRP) cells *in vitro* and *in vivo*.[136] Gefitinib may also enhance the sensitivity of MDR BCRP-overexpressing cancer cells, including human PC-6 small-cell lung cancer cells selected with SN-38 (PC-6/SN2-5H), MCF-7 breast cancer cells selected with mitoxantrone (MCF-7/MXT) or BCRP cDNA-transfected MCF-7 cells to topotecan, SN-38, and mitoxantrone.[141] The use of gefitinib at clinically relevant concentrations also moderately reversed P-gp-mediated resistance to PTX and docetaxel in P-gp-overexpressing MDR PC-6/PTX small-cell lung cancer and MCF-7/Adr breast cancer cells *in vitro*.[138] Similarly, gefitinib also reversed the resistance to PTX in CLI-Pac cells and doxorubicin in MCF-7/Adr cells, which overexpress P-gp and topotecan resistance in BCRP-overexpressing CL1/TPT and MCF-7/TPT cells. However, these effects did not restore the sensitivity of MRP1-overexpressing MCF-7/VP-16 cells to etoposide.[137] This suggests that gefitinib may induce, at least in part, its chemosensitizing effect in MDR cancer cells by inhibiting P-gp and BCRP drug efflux pumps. The coadministration of gefitinib plus a camptothecin derivative, irinotecan (CPT-11), also resulted in an increase of oral bioavailability and tumor growth inhibitory effect induced by irinotecan on a panel of pediatric subcutaneous tumor xenograft models *in vivo*.[140] It has been proposed that this chemosensitizing effect of gefitinib could be mediated, in part, by inhibiting BCRP/ABCG2 transporter activity.

Altogether, these results suggest that the use of EGFR inhibitors, such as gefitinib or CI1033, in combination therapies might have multiple beneficial effects *in vitro* and *in vivo*, including the improvement of both bioavailability and antitumoral activities of combined drugs as well as the reversal of MDR of cancer cells by downregulating ABC multidrug efflux pump expression and activity.

Targeting of ceramide signaling pathways

The ionizing radiation, anti-androgens, and many cytotoxic agents, including the chemotherapeutic drugs, alone or in combination therapy, have been reported to mediate, at least in part, their apoptotic/necrotic effects in diverse human cancer cells by enhancing the cellular ceramide levels.[3,152,163–170] Among them, there are endogenous lipid anandamide, tumor necrosis factor, tumor necrosis factor-related apoptosis-inducing ligand, docetaxel, PTX, etoposide, doxorubicin, irinotecan, gefitinib, and semisynthetic retinoid, *N*-(4-hydroxyphenyl) retinamide (or fenretinide). The molecular mechanisms of cytotoxic effects induced by these agents include the activation of *de novo* ceramide synthesis pathway, acid sphingomyelinase and inhibition of ceramide metabolism (Figure 3). More specifically, the cellular ceramide accumulation in plasma membrane may result in the activation of protein phosphatase 2A and its subsequent translocation to mitochondria where it can contribute to the

inactivation of the Bcl-2 protein.[163] The ceramide may also participate in the formation of large transmembrane channels (Cer channels) in mitochondria. These effects may subsequently result in permeabilizing mitochondrial outer membrane, releasing cytochrome *c* and other proapoptotic factors in cytosol, and activation of caspase-dependent and -independent pathways (Figure 2).[171–173] It has also been observed that the molecular alterations in cellular ceramide pathways may be responsible, in part, for the resistance of certain cancer cells to chemotherapeutic drugs that mediate their cytotoxic effects via the activation of the cellular ceramide accumulation-induced apoptotic death.[163–168,174] For instance, the lack of ceramide generation in response to antimitotic agent, PTX, in the stable human PTX-resistant ovarian carcinoma cell line, CABA-1/PTX, has been associated with PTX resistance.[175] More specifically, the enhanced expression and activity of enzymes involved in the metabolism of proapoptotic sphingolipid ceramide, including the acid ceramidase and sphingosine kinase, which participate in subsequent steps to produce antiapoptotic agent, sphingosine- 1-phosphate, may contribute to intrinsic or acquired drug resistance of cancer cells (Figure 3).[172,175–179] Similarly, the enhanced activity of glucosylceramide synthase (GCS), the enzyme converting ceramide into glucosylceramide, may also decrease the cellular ceramide levels and rate of apoptotic death of cancer cells. Moreover, the enhanced expression and activity of sphingomyelin synthase 1, which catalyzes the conversion of ceramide and phosphatidylcholine into sphingomyelin and diacylglycerolsphingomyelin, or the decreased function of galactocerebrosidase, which is an enzyme that degrades the galactosylcerebroside into ceramide, may also alter the sensitivity of certain cancer cells to cytotoxic drugs (Figure 3).[176,180] On the basis of the major implication of cellular ceramide in triggering the apoptotic death of cancer cells, numerous investigations have been performed for improving the cytotoxic effects of anticarcinogenic drugs by using ceramide metabolism inhibitors to enhance cellular ceramide levels.

Ceramide metabolism inhibitors—Several studies have revealed that the inhibition of the acid ceramidase activity, the enzyme that hydrolyzes ceramide into its metabolite sphingosine and fatty acid by using *N*-oleoylethanolamine, LCL204, or B13, potentiated the cytotoxic effects induced by diverse drugs in metastatic and androgen-responsive and-independent prostate cancer cell lines *in vitro* and *in vivo* (Figures 2 and 3: Table 2).[3,152,181] The MDR associated with doxorubicin and etoposide in HL-60/Doxo and HL-60/VP-16 acute myeloid leukemia cells overexpressing MRP1 and MDR1, respectively, was also reversed by using a specific inhibitor of sphingosine kinase-1, F-12509a.[182] Additionally, it has been reported that the upregulated expression of GCS occurring in diverse human cancer cell lines after exposure to drugs, such as doxorubicin (adriamycin), vinblastine, and etoposide, resulted in high-expression levels of glucosylceramide and P-gp protein concomitant with the acquisition of an MDR phenotype.[179] Interestingly, the inhibition of GCS by small interfering RNA in doxorubicin-resistant MCF7/AdrR breast cancer cells restored their sensitivity to the DNA-interacting drug, doxorubicin, and other antineoplastic drugs, vinblastine and PTX.[183,184] Importantly, the down-regulation of GCS by interfering RNA or using D,L-*threo*-1-phenyl-2-palmitoylamino-3-morpholino-1-propanol, a potent chemical inhibitor of GCS, also decreased MDR1 expression and enhanced the uptake and cytotoxic effects of chemotherapeutic drugs (Figure 3).[183] It is noteworthy that the P-gp transporter modulators such as verapamil, cyclosporin A, and tamoxifen have also been reported to enhance ceramide levels in cancer cells by downregulating the glucosylceramide formation, whereas other agents such as PSC833 may act by stimulating *de novo* ceramide synthesis pathway (Figure 3 and Table 2).[121,124,152,172,176,185–187] Therefore, it is likely that these MDR ABC transporter inhibitory agents may also induce their chemosensitizing effect by promoting the cellular ceramide accumulation-induced apoptotic death.

Hence, in light of these observations, it appears that the alterations in ceramide metabolism in cancer cells may be a determinant factor involved in the MDR phenomenon associated with

the use of chemotherapeutic drugs. Moreover, the increase of cellular ceramide levels induced by different cytotoxic agents may also be accompanied simultaneously by downregulating expression or modulation of certain ABC multidrug efflux pumps. The molecular events that are associated with the crosstalk between the ceramide and ABC transporter signaling are not precisely known. However, it is likely that the downregulation of ABC transporter expression induced by certain modulators of ceramide metabolism may contribute, at least in part, to their chemosensitizing effect in particular cancer cell types and vice versa.

Functions of cancer progenitor cells in cancer progression and novel targeting therapies

Numerous lines of evidence indicated that the leukemic or tumorigenic cancer progenitor cells with the stem cell-like properties can assume a critical role by driving leukemia or tumor formation and generating a heterogeneous population of further differentiated cancer cells in primary neoplasms. [3,12,13,15,21,27,29,32,9–45,188–190] The activation of different oncogenic signaling pathways in cancer progenitor cells and their early progeny, as well as the changes in their local microenvironment may also result in their acquisition of a more aggressive phenotype during the progression from localized cancer into invasive and metastatic disease states. More specifically, the tumorigenic cancer progenitor cells may give rise to poorly to moderately differentiated progeny and acquire a migratory behavior during the epithelial–mesenchymal transition program in primary neoplasm. [12,15,39,51,52,92,191] The cancer progenitor cells also possess some intrinsic properties in common with adult stem cells that may allow them to remain in a quiescent and viable state of dormancy under the specific microenvironmental conditions prevalent at the primary and distant metastatic sites after therapeutic treatments.[12,13,15,39,43,49,57,58] Consistent with this, several studies revealed that the enhanced expression of stem cell-like gene products in cancer progenitor cells is generally associated with a poor prognosis of patients with different cancer types. Particularly, the sustained activation of EGFR, hedgehog, Wnt/β-catenin, and integrins in cancer progenitor cells during cancer progression may lead to an enhanced expression of several oncogenic gene products, such as matrix metalloproteinases, urokinase plasminogen activator, COX-2, vascular epidermal growth factor, and transcription repressors of E-cadherin (snail and slug). These signaling elements may contribute to their unlimited growth and survival and also their resistance to current therapeutic treatments (Figure 1).[12,13,30,31,39,51,52,57,58,192] These oncogenic events are consistent with the cancer stem/progenitor cell model of carcinogenesis, in which accumulating genetic alterations leading to a deregulated activation of diverse oncogenic cascades by multiple growth factors in cancer progenitor cells and their early progeny may serve critical functions for leukemic graft or tumor growth, metastasis, and disease relapse.[12,13,15,21,33,34,39,193,194]

In addition, certain established cancer cell lines derived from brain, lung, liver, breast, ovarian, prostate, and gastrointestinal tumors and maintained in culture for a long period of time may also represent a heterogeneous population of cancer cells. It has been observed that only a small sub-population may correspond to highly leukemic or tumorigenic cancer progenitor cells with the stem cell-like properties. This small sub-population of cancer progenitor cells may be isolated from well-established cell lines by fluorescence-activated cell shorting technique based on their expression of specific stem cell-like surface markers, such as CD34, Sca-1, CD133, and CD44 as well as their high ability to efflux fluorescent Hoechst dye compared with other cancer cell populations.[21,50,97,100,104] In this regard, this very small sub-population of cancer progenitor cells generally expresses the higher levels of stemness genes, such as ABC multidrug efflux transporters, EGFR, hedgehog signaling elements, and β-catenin, than the bulk mass of differentiated cancer cells. Additionally, this very small sub-population of cells is generally more leukemic or tumorigenic and is able to generate leukemic grafts or tumors *in vivo* at a low number of cells compared with their further differentiated progeny. More specifically, a small cell sub-population designated as side population (SP) cells, which

generally possesses stem cell-like properties, including a high Hoechst dye efflux capacity, because of an elevated expression levels of ABC multidrug efflux pumps relative to non-SP fraction, has been detected in diverse mammalian cancer cell lines and cancer cells from tumor tissues (Table 2).[46,47,50,189,195–201] It has been reported that SP cells, but not non-SP cells, could generate both SP and non-SP cells in culture by asymmetric division and were largely responsible for the *in vivo* leukemic graft or tumor formation established from these cancer cell lines in animal models *in vivo*. Similarly, the *in vitro* and *in vivo* characterization of $CD44^{+/high}$-positive prostatic cancer cells LAPC-4, -9, DU145, and PC3 cells isolated from the total parental cell population revealed that this cancer cell sub-population was more proliferative, invasive, and tumorigenic than the CD44-negative or $CD44^{low}$-positive cancer cell sub-population. [188,190] Taken together, these observations emphasize the importance of considering the presence of this small sub-population of more leukemic or tumorigenic cancer progenitor cells when these cancer cell lines are used to test the anticarcinogenic effects of novel combinations of drugs. This should allow us to obtain a better estimation of efficacy of antitumoral drugs to eliminate the cancer-initiating cells found in available cancer cell lines.

Drug resistance of cancer progenitor cells

Several recent lines of evidence have indicated that a small population of leukemic or tumorigenic cancer progenitor cells possessing stem cell-like properties may be more resistant to chemotherapy than their further differentiated progeny. More specifically, high-expression levels of ABC multidrug efflux transporters and antiapoptotic signaling factors (Bcl-2, EGF–EGFR, and sonic hedgehog (SHH)-PTCH) and alterations in tumor suppressor gene products such as *p*53, combined with the active DNA repair mechanisms in cancer progenitor cells, may confer on them their intrinsic or acquired resistance to hormonal therapies, radiotherapy, and chemotherapeutic drugs (Figure 1 and Table 1).[21,24,86,97,98,100,101,202,203] Regardless, the occurrence of different genetic or epigenic alterations, including mutations and the activation of different oncogenic cascades in cancer progenitor cell sub-populations during cancer initiation and progression, may be partly responsible for the intratumoral heterogeneity or diversity of cancer subtypes. [12,13,33,39,45,52,81–83,193,204] In particular, the acquisition of mesenchymal and migratory properties by poorly differentiated or moderately differentiated cancer progenitor cells expressing the basal or intermediate epithelial cell markers, respectively, during the epithelial–mesenchymal transition program may result in highly aggressive cancer forms.[12,39,44,45,51,52,165,199,203–206] Of critical therapeutic importance, these different cancer-initiating progenitor cell subsets, with different mutations and oncogenic properties, may also respond with different sensitivity to cytotoxic drug treatment. Thereby, they can contribute to the partial response or resistance to current therapies observed in different subsets of patients.[44,45,52,165,199,203–209] This new model on the heterogeneity of cancer progenitor cells has important repercussions for the design of new effective diagnostic methods and preventive and curative treatments. Indeed, the model implicates that specific therapeutic regimens may be effective to eradicate only certain leukemic or tumorigenic cancer progenitor cells within a cancer subtype, whereas other sub-populations of cancer progenitor cells may be more resistant to treatments. For instance, the malignant transformation of the most primitive ERα-negative cancer progenitor cells may result in the formation of poorly differentiated and aggressive tumor types with a basal phenotype.[204] Hence, the persistence of ERα-negative cancer progenitor cells may contribute to resistance to anti-estrogen therapy and disease relapse.[204] In contrast, the enhanced expression of ERα in cancer progenitor cells in other breast cancer subtypes may represent a determinant factor that may influence their greater sensitivity and response to selective anti-estrogen therapies, including tamoxifen and aromatase inhibitors, than their clinical inefficacy against ERα-negative cancer cells. Furthermore, it has been observed that the expression levels of CD133 biomarker, which may be associated with the presence of CD133-positive cancer progenitor cells, were significantly higher in recurrent glioblastoma multiforme (GBM) tissues obtained from five patients than

those detected in newly diagnosed tumor tissues from the same patients.[203] The reverse transcription-polymerase chain reaction analyses of gene expression profile for CD133-positive cancer progenitor cells found in three primary cultured cell lines established from GBM patients have also indicated that these cancer progenitor cells express higher levels of diverse stemness genes, including certain gene products related to drug resistance, than the autologous CD133-negative cells.[203] Among the stem cell-like gene products are Oct-4 and CD44 biomarkers, BCRP/ABCG2 transporter, the DNA repair protein O6-methylguanine-DNA methyltransferase, antiapoptotic factors, including Bcl-2, survivin, inhibitor of apoptosis proteins, survival signaling elements SHH/PTCH/GLI, and the transcriptional repressor of the E-cadherin snail.[203] Additionally, the CD133-positive cancer progenitor cells from GBM tissues were significantly more resistant to chemotherapeutic agents, including oral DNA alkylating agent, temozolomide, carboplatin, etoposide, and PTX, than the autologous CD133-negative cancer cells.[203] This suggests that the persistence of these CD133-positive cancer progenitor cells may partly contribute, to the recurrence of highly aggressive GBMs. Importantly, it has recently been reported that the ABC transporters did not appear to contribute to the resistance of GBM stem cells to several chemotherapeutic drugs. In fact, other MDR mechanisms, such as the alterations in apoptotic signaling cascades, including the overexpression of antiapoptotic factors, could represent a more important factor that confers on them their chemoresistance.[210] It is noteworthy that the GBMs, which appear to derive from the malignant transformation of neural stem cells that acquire mesenchymal properties like mesenchymal stem cells, are frequently accompanied by the overexpression of EGFR.[44,45,52] The enhanced EGFR expression may then contribute, at least in part, to their survival, acquisition of more aggressive behavior, and chemoresistance. Interestingly, a subset of tumorigenic progenitor cells designated as tumorospheres and possessing an angiogenic potential and MDR phenotype, including the expression of MRP1 and -3, has also been identified in human glioblastoma. This suggests that this cancer cell population may contribute to drug resistance.[211] In addition, the resistance of CD133-positive glioma progenitor cells to radiation may also be due to the activation of DNA damage checkpoint response and DNA repair mechanisms.[205] In contrast, it has been reported that the BCR-ABL kinase inhibitor, imatinib mesylate, can also act as an inhibitor of BCRP/ABCG2 transporter, which is over-expressed in chronic myelogenous leukemia (CML) $CD34^+$ hematopoietic progenitor cells.[133] However, it appears that non-proliferative CML $BCR\text{-}ABL^+$ $CD34^+$ progenitor cells may be more resistant to cytotoxic effects induced by diverse agents, including imatinib mesylate, cytosine arabinoside, and arsenic trioxide, than the dividing $BCR\text{-}ABL^+$ $CD34^+$ progenitor cells.[165,206,209] Similarly, it has been observed that the mammospheres of non-adherent $CD44^+/CD24^{-/low}$ breast cancer stem cells established after their isolation from MCF-7 breast cancer cell line were more resistant to radiation than adherent cancer cells grown as monolayer cultures, and their number was enhanced after treatment with the fractionated doses of irradiation.[208] Hence, the persistence and increase of the number of cancer-initiating cell population after treatment with these types of anticarcinogenic treatment may partly contribute to disease relapse.

Additionally, the increased expression levels and activity of diverse ABC transporters and survival factors have also been observed in the SP fraction relative non-SP cell sub-population. For instance, the expression of MDR1/P-gp, BCRP/ABCG2, and CEACAM6, which are associated with multidrug chemoresistance to anticancer agents, was significantly increased in SP cells versus non-SP cells isolated by the drug efflux Hoechst dye technique from hepatoma HuH7 and colorectal SW480 cancer cell lines.[199] HuH7 SP cells also displayed a higher resistance to doxorubicin, 5-fluorouracil, and gemcitabine than non-SP cells.[199] Interestingly, a SP cell population has also been detected in neuroblastoma cells from 15 of 23 patients (65%), which expressed high levels of BCRP/ABCG2 and ABCA3 transporter genes and a greater capacity to efflux cytotoxic drugs such as mitoxantrone than a non-SP cell sub-population.[50] The ABCB5 transporter, which is expressed in human malignant melanoma cells

with a $CD133^+$ stem cell-like phenotype, may also mediate the enhanced doxorubicin efflux transport and chemoresistance. [207] In addition, the results from recent studies have revealed that EGF may induce the upregulation of ABC transporters in certain normal cells and enhanced SP cell fraction in human metastatic head and neck squamous cell carcinoma cell lines, UMSCC10B and HN12 cells expressing BCRP/ABCG2 transporter.[201,212] Importantly, it has also been observed that in the treatment of head and neck squamous cell carcinoma cell lines by EGFR inhibitor, gefitinib decreased the cell number in the SP population, which was associated with a decrease of the ABC transporter on the cell surface.[201] This suggests that the activation of EGF–EGFR pathway in cancer progenitor cells could contribute to the enhancement of membrane ABC transporter levels and thereby contribute to MDR phenomenon. Further studies on the regulatory mechanisms of expression and activity of ABC transporters in MDR cancer progenitor cells versus parental drug-sensitive cancer cells should allow us to confirm the implication of growth factors, including the EGF–EGFR system, in the control of ABC pump expression in other cancer progenitor cell types.

Targeting of cancer progenitor cells

The developmental signaling cascades (EGFR, hedgehog, and Wnt/β-catenin), oncogenic signaling elements (telomerase, Scr, Bcl-2, nuclear factor-κB, PI^3K/AKT, and COX-2), and ABC multidrug efflux pumps may assume a critical function in regulating the stem cell self-renewal, differentiation, survival, and drug resistance of cancer stem/progenitor cells.[2,13,15,21,24,30,31,39,49,50,92,152,153,203,213–217] Therefore, the molecular targeting of these signaling elements, which are activated in certain leukemia subtypes, multiple myeloma, and numerous solid cancers, is of particular therapeutic interest (Table 2).[1] Regardless, the recent development of diverse enhanced delivery techniques for administration of anticarcinogenic drugs may constitute promising approaches for targeting cancer progenitor cells, which express specific biomarkers and thereby reverse MDR *in vivo*. Among them are the targeted delivery of therapeutic agents into tumors by using the conjugation/fusion of drugs to tumor-specific antibodies, encapsulation of chemotherapeutic drugs in liposomes or other carriers such as nanoparticules, and the use of genetically engineered stem cells as vehicles.[114,218–220] These delivery techniques may enhance drug bioavailability, improve selectivity, and continue delivering drugs into particular tumoral tissues, thereby decreasing the systemic toxicity.[114,218–220]

Certain investigations have also revealed that the use of combined pharmacological agent that are able to induce the differentiation and apoptotic death of leukemic or tumorigenic cancer progenitor cells may constitute effective therapeutic treatment or adjuvant therapy.[221–223] For instance, the use of a highly effective therapeutic agent, imatinib mesylate, a TKI-active against BCR-ABL oncoprotein, generally leads to a clinical remission in the early chronic phase of CML.[224–226] Although BCR-ABL inhibitors, including imatinib mesylate and dasatinib (BMS-354825), may be effective in eradicating the dividing $BCR\text{-}ABL^+$ CML cells, the persistence of non-proliferative $BCR\text{-}ABL^+$ CML cells expressing lower level of BCR-ABL oncoprotein, which may be more resistant to apoptotic effect of these agents, may subsequently lead to disease relapse.[165,206,209,224,227] Additionally, the progression to the advanced- and late-stage disease, accelerated phase, or terminal blast crisis phase of CML disease is often accompanied by acquisition of a more malignant phenotype and a lack of response to BCR-ABL inhibitors concomitant with the leukemia recurrence.[224–226] The inhibition of hedgehog and Wnt/β-catenin cascades, antiapoptotic factor Bcl-2, and Scr-related LYN kinase, which are activated in certain patients during CML progression and the cellular organic cation transporter OCT-1 involved in the intracellular uptake of imatinib, may represent promising adjuvant strategies (Table 2).[227–230] Of particular therapeutic interest, recent *in vitro* studies revealed that the exposure of primitive quiescent $BCR\text{-}ABL^+$ CML progenitor cells to growth factors such as granulocyte colony-stimulating factor and granulocyte macrophage colony-

stimulating factor at clinically achievable concentrations may reduce the number of non-dividing BCR-ABL$^+$ CML progenitor cells.[231,232] This effect may thereby overcome the resistance to imatinib mesylate and promote the elimination of residual malignant CML cells.[231,232] Interferon-α also appears to be more effective than imatinib mesylate in eliminating the primitive BCR-ABL$^+$ CML progenitor cells, which are responsible for the maintenance of long-term cultures, whereas imatinib mesylate could be more active against the further differentiated CML progenitor cells.[233] In fact, the antileukemic activity of interferon-α may be mediated, in part, by inducing a differentiating effect on primitive CML progenitors.[233] Thus, despite the fact that clinical therapeutic effects by using interferon-α plus stem cell transplantation are generally less rapid and effective after the initiation treatment than imatinib mesylate, the response could be more stable after discontinuation of the drug. Therefore, this therapy could represent certain clinical advantages for long-term care of certain patients who did not respond to treatment with BCR-ABL inhibitors. Similarly, the use of differentiating agent, all-*trans* retinoic acid with chemotherapeutic drugs such as anthracyclines has resulted in a successful treatment of acute promyelocytic leukemia, which is associated with the expression of the PML-RAR-α^+ oncoprotein produced from the fusion of the promyelocytic leukemia (*PML*) gene to retinoic acid receptor α (*RAR-α*) gene.[234,235] The combination of all-*trans* retinoic acid-based therapy with other anticancer drugs, including arsenic trioxide, which can induce the antiproliferative and proapoptotic effect on PML-RAR-α^+ acute promyelocytic leukemia cells, including leukemic stem cells, may represent a more promising approach to treat acute promyelocytic leukemia. The advanced or refractory/relapsed acute promyelocytic leukemia or other cancer types such as multiple myeloma are also sensitive to these agent types.[222,234–236]

CONCLUSIONS AND PERSPECTIVES

Together, these recent investigations revealed that high resistance to current therapeutic treatments may result from several different structural and functional properties between the cancer progenitor cells and their further differentiated progeny, including certain molecular mechanisms that may contribute to their high resistance to current therapeutic treatments. Further characterization of cancer progenitor cells versus their more differentiated progeny and normal counterpart adult stem cells should lead to the identification of new drug targets for the development of more effective strategies for the prevention and curative treatment of aggressive cancers. Additional analyses of oncogenic gene expression profiles and drug resistance properties specific to tumorigenic and migrating cancer progenitor cells should allow us to selectively target this minority of cancer-initiating cells for improving the current clinical therapies against the most metastatic and incurable cancers. The molecular targeting of ABC multidrug efflux transporters, EGFR, hedgehog, and Wnt/β-catenin, and deregulated apoptotic signaling that provide a critical function for the sustained growth, survival, metastasis, and development of chemoresistance of cancer progenitor cells should lead to improved treatments for highly aggressive and metastatic cancers. In this regard, it will be important to more precisely establish the molecular mechanisms associated with the MDR inhibitory effect induced by specific or broad ABC multidrug transporter inhibitors, EGFR inhibitor such as gefitinib and ceramide metabolism inhibitors, alone or in combination, on isolated leukemic or tumorigenic cancer progenitor cells. The studies carried out with the cancer progenitor cells made resistant to drugs of clinical interest *in vitro* and *in vivo*, and subsequently in clinical settings, merit further investigations. Hence, further works should lead to development of novel strategies for counteracting the MDR phenomenon that occurs specifically in leukemic or tumorigenic cancer progenitor cells and thereby improve current therapeutic treatments against recurrent cancers and long-term survival of patients.

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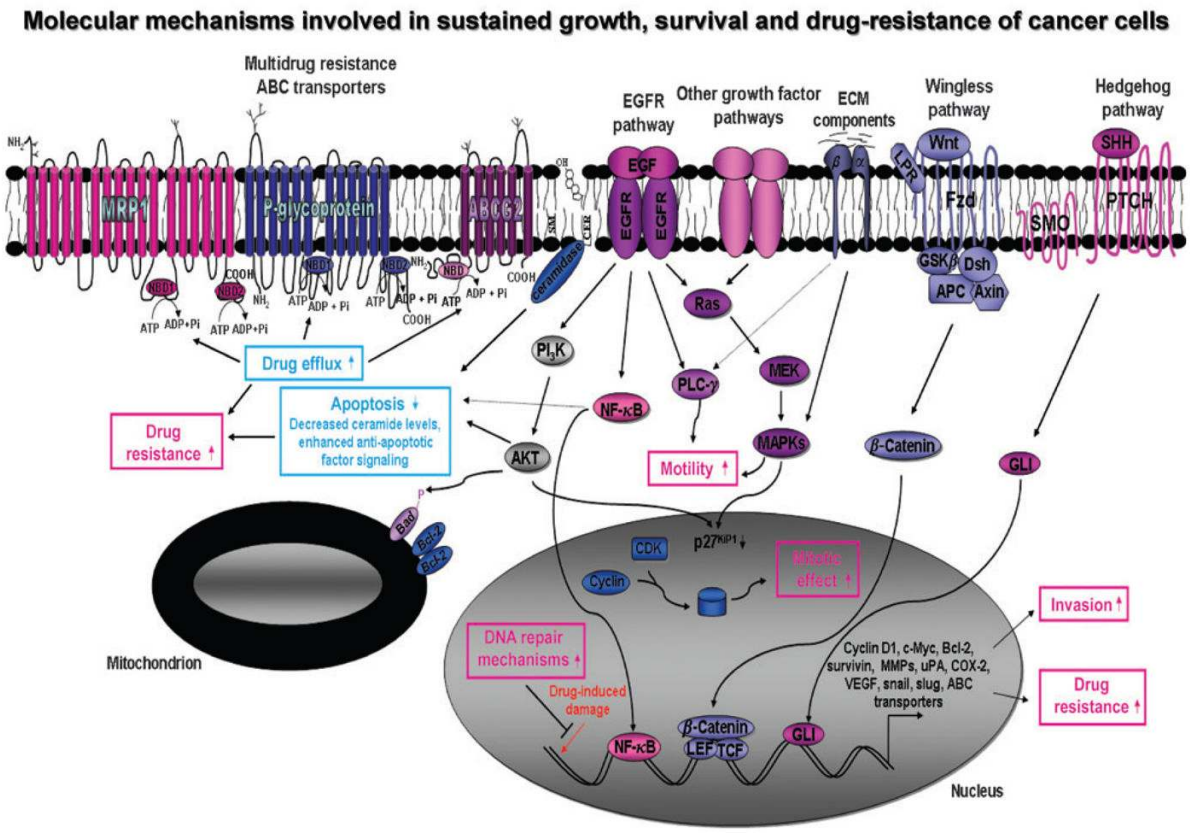

Figure 1.

Schematic showing the possible molecular mechanisms involved in the sustained growth, survival, invasion, and drug resistance of cancer cells. The oncogenic intracellular cascades induced through the activation of distinct growth factor signaling pathways, including EGF–EGFR system, sonic hedgehog SHH/PTCH/GLI, Wnt/*β*-catenin, and extracellular matrix (ECM) component/integrins, which may provide a critical role for the sustained growth, survival, and invasion of cancer cells, are shown. The upregulated expression levels of certain target gene products, including upregulated antiapoptotic factors Bcl-2 and survivin, matrix metalloproteinases (MMPs), urokinase plasminogen activator (uPA), cyclooxygenase (COX-2), vascular epidermal growth factor (VEGF), transcriptional repressor of E-cadherin (snail and slug), and MDR ABC transporters that can contribute to the malignant transformation of cancer cells and drug resistance are indicated. In addition, the possible drug efflux via the MDR ABC transporters, including MRP1, P-glycoprotein, and brain cancer resistance protein (BCRP/ABCG2), is also shown. APC, adenomatous polyposis coli; CDK, cyclin-dependent kinase; CER, ceramide; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; FZD, Frizzled receptor; IGF, insulin-like growth factor; IGF-1R, insulin-like growth factor-1 receptor; LEF, lymphoid enhancer factor; LPR, low-density lipoprotein receptor-related protein; MAPKs, mitogen-activated protein kinase; MEK, extracellular signal-related kinase kinase; NBD, nucleotide binding domain; NF-*κ*B, nuclear factor-*κ*B; PI_3K, phosphatidyl inositol 3′-kinase; PLC-*γ*, phospholipase C-*γ*; SHH, sonic hedgehog; SM, sphingomyelin; SMO, smoothened; TCF, T-cell factor; uPA, urokinase plasminogen activator; Wnt, wingless.

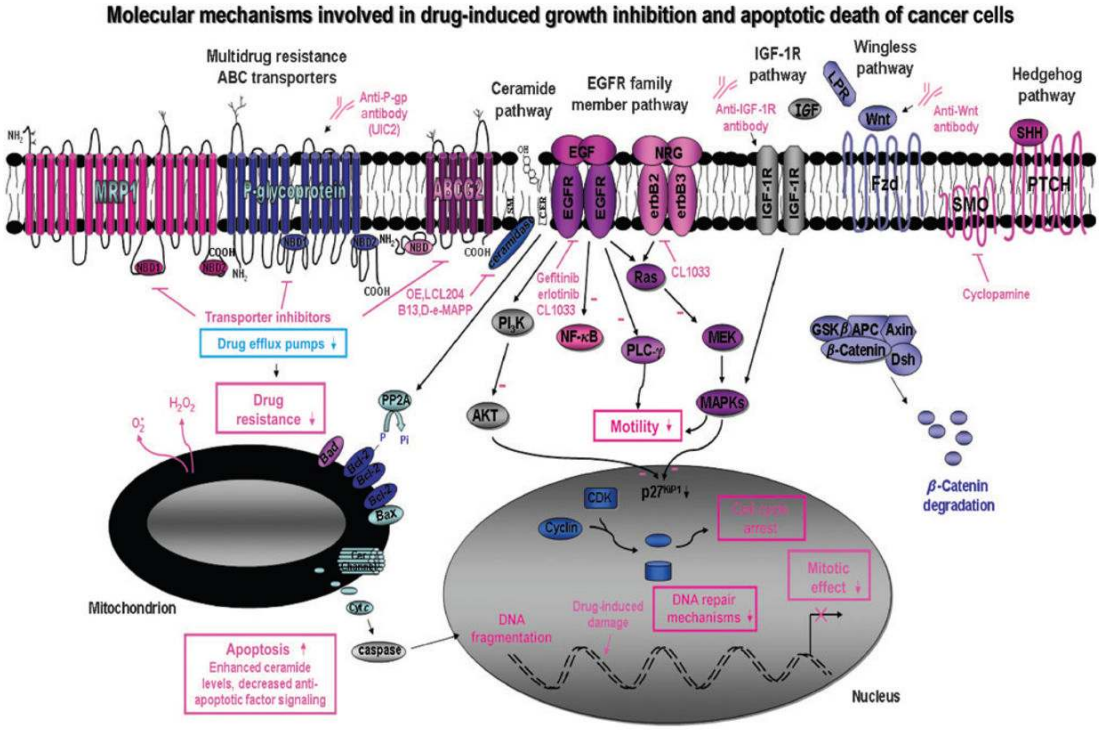

Figure 2.

Proposed molecular targets in cancer cells for the development of novel therapeutic strategies against aggressive and recurrent cancers. The schematic shows the possible antiproliferative and apoptotic effects induced by the tyrosine kinase activity inhibitors, including EGFR (gefitinib and erlotinib) and EGFR/erbB2/erbB3 (CI-1033), as well as by a selective inhibitor of SMO hedgehog signaling element (cyclopamine) and monoclonal antibody directed against Wnt and IGF-1R. Moreover, the reversal of the MDR mediated through ABC transporters by using specific transporter inhibitors and antibody directed against P-glycoprotein drug efflux pump is also shown. Cyt *c*, cytochrome *c*; PP2A, protein phosphatase 2A.

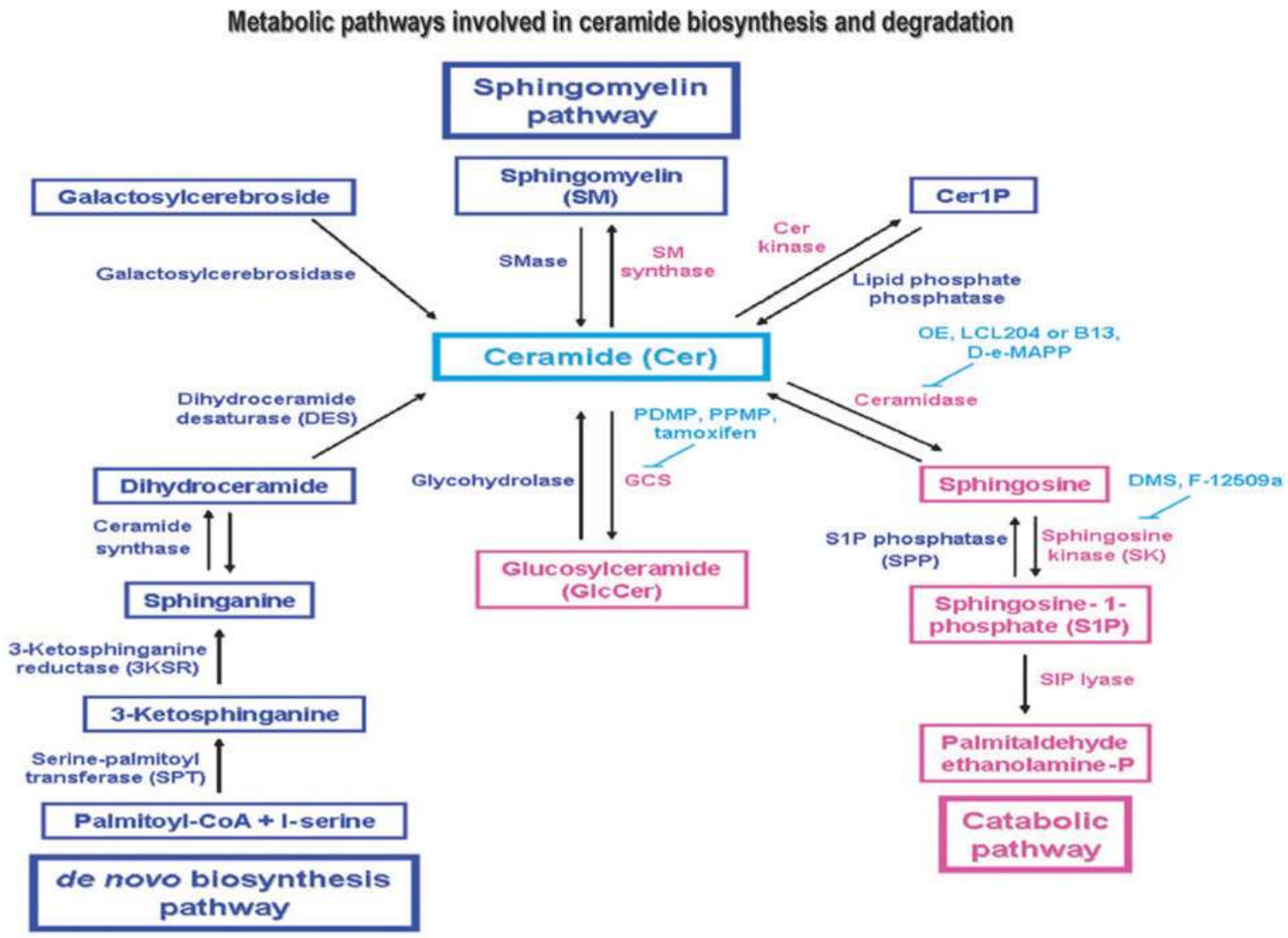

Figure 3.

Schematic representation of metabolic pathways involved in the biosynthesis and degradation of the endogenous sphingolipid ceramide. The cellular ceramide (Cer) accumulation induced through the activation of *de novo* synthesis and sphingomyelin pathways as well as the catabolic pathway involved in ceramide degradation are shown. Moreover, the blockade of the ceramide degradation by using the specific inhibitors of the enzymes that catalyze the transformation of proapoptotic ceramide into its diverse metabolites, including ceramidase, sphingosine kinase, and GCS inhibitors, and whose inhibitory agents can induce the ceramide accumulation-induced apoptotic death in cancer cells are also indicated. D-e-MAPP, D-erythro-2-(*N*-myristoylamino)-1-phenyl-1-propanol; DMS, *N*,*N*-dimethylsphingosine; GCS, glucosylceramide synthase; OE, *N*-oleoylethanolamine; PDMP, D,L-*threo*-1-phenyl-2-decanoylamino-3-morpholino-1-propanol; PPMP, 1-phenyl-2-palmitoylamino-3-morpholino-1-propanol.

Table 1

MDR ABC transporter superfamily members and their cytotoxic drug resistance profile

Transporter/subfamily name	Cytotoxic drug resistance profile
ABCA2	Estramustine, mitoxantrone (MXT), doxorubicin (Doxo) or adriamycin (Adr), methotrexate, vinblastine
ABCA3	Doxorubicin, methotrexate, vinblastine
MDR1 (P-gp)/ABCB1	Anthracyclines (doxorubicin, daunorubicin, epirubicin), actinomycin D, colchicine, mitomycin C, epipodophyllotoxin derivatives (etoposide (VP-16), teniposide), methotrexate, mitoxantrone, taxanes (paclitaxel (PXT or taxol), docetaxel (taxotere)), *Vinca* alkaloids (vincristine, vinblastine)
MRP1/ABCC1	Anthracyclines, colchicine, etoposide, methotrexate, paclitaxel, camptothecine, vincristine, vinblastine
MRP2/ABCC2	Irinotecan (camptothecin-11, CPT-11, or camptosar), active metabolite of irinotecan (7-ethyl-10- hydroxycamptothecin, SN-38), cisplatin, doxorubicin, etoposide, methotrexate, vincristine, vinblastine
BCRP/ABCG2	Anthracyclines, bisantrene, 9-amino-camptothecin, irinotecan, SN-38, topotecan (TPT), mitoxantrone, methotrexate, etoposide, epirubicin, flavopiridol

ABC, ATP-binding cassette; BCRP, breast cancer resistance protein; MDR1, multidrug resistance 1; MRP1, multidrug resistance-associated protein 1; MRP2, multidrug resistance-associated protein 2; P-gp, P-glycoprotein.

Table 2

Inhibitory agents targeting deregulated signaling elements involved in sustained growth, survival, and drug resistance of cancer cells

Targeted signaling element	Name of inhibitory agent
Growth and survival signaling	
EGFR family member inhibitors	
Anti-EGFR (erbB1) antibody	mAb-C225, IMC-C225
Anti-EGF antibody	ABX-EGF
Antisense oligonucleotide	As-EGFR, As-EGF, As-TGF-α
EGFR-TKI	AG1478, gefitinib, erlotinib, EKB-569
Anti-erbB2 antibody	Trastuzumab
EGFR-ErbB2-TKI	PKI-166, TAK165, GW572017 (lapatinib)
erbB1, erbB2, erbB3, erbB4-TKI	CI1033
EGFR/VEGFR-TKI	AEE788, ZD6474
Other growth factor signaling inhibitors	
Hedgehog signaling inhibitor	SMO inhibitor cyclopamine, anti-SHH antibody
Wnt signaling inhibitor	Anti-Wnt antibody, WIF-1
Notch signaling inhibitor	γ-secretase inhibitor
IGF-1R signaling inhibitor	Adenovirus-IGF-1r/dn, anti-IGF-1R antibody (A12), IGF-1R-TKI (NVP-AEW541)
BCR-ABL-TKI	Imatinib mesylate (ST1571), dasatinib, nilotinib
Src-family and ABL-TKI	PD180970
Src-family-TKI	CGP-76030
Hormonal signaling inhibitors	
AR	Bicalutamide, flutamide
ER	Tamoxifen, raloxifen
Apoptotic signaling activators	
Ceramide generation activator	Docetaxel, paclitaxel, etoposide, doxorubicin, gefitinib,
Ceramide synthase activator	PSC833
Ceramidase inhibitor	*N*-oleoylethanolamine (OE), LCL204, B13, D-e-MAPP
GCS inhibitor	D,L-*threo*-PPMP (PDMP), PPMP, tamoxifen
Sphingosine kinase-1 inhibitor	*N,N*-dimethylsphingosine (DMS), F-12509a
Bcl-2 inhibitor	As-Bcl-2, ABT-737
PI_3K inhibitor	LY294002, rapamycin, CCI-779
NF-κB inhibitor	IκBα inhibitor, sulfasalazine, bortezomib (PS-341)
COX-2 inhibitor	NS-398, etodolax, celecoxib, rofecoxib
VEGFR inhibitor	Anti-VEGFR-antibody, SU5416
Drug resistance signaling inhibitors	
ABC transporter inhibitors	
P-gp/MDR1/ABCB1	UIC2 monoclonal antibody, antisense oligodeoxynucleotide verapamil, dexverapamil, cyclosporin A, valspodar (PSC-833), quinidine, cinchonine, tamoxifen, toremifene, VX-710 and GF-120918, retinoid X receptor-selective agonist bexarotene (LGD1069, Targretin), dofequidar fumarate, MS-209, VX-710, flavonoids, gefitinib, CI1033

Targeted signaling element	Name of inhibitory agent
MRP1/ABCC1	Anti-MRP1 antibody, NSAIDs (indomethacin, sulindac, tolmetin, acemetacin, zomepirac, mefenamic acid), quinidine, MS-209, VX-710, flavonoids
BCRP/ABCG2	Flavonoids, tamoxifen derivatives, gefitinib, C11033, Prazosin
Organic cation transporter OCT-1 inhibitor	
Drug resistance signaling inhibitor	
EGFR-TKI-DR	IGF-1R inhibitor
AR/anti-androgen-DR	EGFR inhibitor (gefitinib)
ERα/anti-estrogen-DR	EGFR inhibitor (gefitinib), erbB2 inhibitor

ABC, ATP-binding cassette; AR, androgen receptor, BCRP, breast cancer resistance protein; COX, cyclooxygenase; D-e-MAPP, D-erythro-2-(*N*-myristoylamino)-1-phenyl-1-propanol; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ER, estrogen receptor; GCS, glucosylceramide synthase; IGF-1R, insulin-like growth factor-1 receptor; IκBα, inhibitor κBα; mAb, monoclonal antibody; MDR1, multidrug resistance 1; MRP1, multidrug resistance-associated protein 1; NF-κB, nuclear factor-κB; NSAIDs, non-steroidal anti-inflammatory drugs; OCT-1, organic cation transporter- 1; P-gp, P-glycoprotein; PI3K, phosphoinositide-3 kinase; SHH, sonic hedgehog; SMO, smoothened; TKI, tyrosine kinase inhibitor; VEGFR, vascular epidermal growth factor receptor; WIF-1, Wnt inhibitory factor-1; Wnt, wingless.