Molecular Cancer Therapeutics: Recent Progress and Targets in Drug Resistance

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Abstract

Recent progress in development of molecular cancer therapeutics revealed new types of antitumor drugs, such as Herceptin, Gleevec, and Iressa as potent therapeutics for each specific tumor. We have been working on molecular cancer therapeutics, and in particular, those related to drug resistance, Here, I describe several resistance mechanisms, including apoptosis regulation, cellular stress response and cellular survival signals which have show close relevance to drug resistance. P-glycoprotein (P-gp) is the key molecule in multidrug resistance (MDR) and a good target for chemotherapy. Proteasome is involved in the resistance mechanism to topo II-targeted chemotherapy in solid tumors. Apoptosis program in tumor cells plays a critical role in chemotherapy-induced tumor cell killing, and the blockade of the apoptosis-inducing pathway could be another mechanism for drug resistance. Glyoxalase I is a molecule involved in apoptosis resistance mechanism in tumors. Survival (antiapoptosis) signals are the good targets for various antitumor drugs to overcome innate drug resistance. Our present studies provide novel targets for effective molecular cancer therapeutics in future.
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Key words: drug resistance, apoptosis resistance, proteasome, glyoxalase I, AKT

Introduction

We have been working in the development of molecular cancer therapeutics in the field of drug resistance. The effectiveness of chemotherapy has been often limited by drug resistance of tumors. Because of the serious problem of clinical drug resistance, much effort has been extended to advance our understanding of the mechanism of drug resistance in cancer cells. The molecular mechanisms of drug resistance, however, are not yet fully understood. P-glycoprotein (P-gp), encoded by MDR1 gene, is a multidrug transporter and has a major role in multidrug resistance (MDR). Targeting of P-gp by small molecule compounds and/or antibodies is an effective strategy to overcome MDR in cancer, especially in hematologic malignancies. Several P-gp inhibitors are developed and are currently under clinical phase studies.

Proteasome is a major intracellular machinery for protein degradation, and is often involved in cellular stress response. The pathological stress conditions, such as glucose deprivation and hypoxia, are common features of solid tumors and play a role in developing drug resistance. Indeed, these conditions cause decreased expression of DNA topoisomerase IIa (topo IIa), rendering stressed cells resistant to topo II-targeted drugs, such as etoposide and doxorubicin. We found that inhibition of proteasome suppressed the stress-induced topo IIa depletion, and prevented the etoposide resistance *in vitro* and *in vivo*. Thus, proteasome can be a new therapeutic target for circumventing resistance to topo II-targeted chemotherapy in solid tumors.

Apoptosis and anti-apoptosis pathways are also deeply related to drug sensitivity and resistance. Many tumor cells have been reported to undergo apoptotic cell death when treated with etoposide, camptothecin, cisplatin, 1-β-D-arabinofuranosyl cytosine (Ara-C), mitomycin C, adriamycin, and vincristine. Apoptosis program in tumor cells plays a critical role in chemotherapy-induced tumor cell death, and inhibition of the apoptosis-inducing pathway could be another mechanisms of multidrug resistance. We found that glyoxalase I (GL01), an enzyme that detoxifies methylglyoxal, is selectively overexpressed in the apoptosis-resistant UK711 cells. Inhibitor of GL01 enhanced etoposide-induced apoptosis in resistant UK711 cells but not in parental U937 cells. GL01 activity was frequently elevated in human lung carcinoma cells. The inhibitor significantly inhibited the growth of xenografted DMS114 and human prostate cancer DU-145. The present results indicate that GL01 is a tumor-specific target enzyme, especially in human lung carcinoma cells and that the GL01 inhibitor is a potent

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chemotherapeutic agent to supress GL01-overexpressing human tumors.

Further, Serine/threonine kinase Akt is thought to mediate many biological actions toward anti-apoptotic responses. Screening of drugs that could interfere with the Akt signaling pathway revealed that UCN-01 (7-hydroxystaurosporine), a drug now in clinical trials and with a unique fingerprint pattern, induced Akt dephosphorylation, which resulted in Akt inactivation and apoptosis of the cells. Further analysis revealed that UCN-01-mediated Akt inactivation was caused by inhibiting upstream Akt kinase PDK1 both *in vitro* and from cells. Because we discovered that other anti-tumor drugs, such as Topotecan, Geldanamycin, and Radicicols, also activated apoptosis by inhibiting the Akt signaling pathway, PDK1-Akt survival pathway is a new, attractive target for cancer chemotherapy.

Based on the drug- and apoptosis-resistant mechanisms described above, we can design a rational strategy to target resistant cancers. Our studies could provide new molecular cancer therpeutics in future. In the following sections, I would like to describe the progress in molecular cancer therapeutics developed clinically and our basic approach for the future development of molecular therapeutics.

Recent Progress in Molecular Cancer Therapeutics

Recent progress in molecular cancer therapeutics has revealed several promissing drugs and therapeutic approaches. One such approach is the use of inhibitors for matrix metallo protease (MMP). MMPs are essential proteases involved in tumor invasion and metastasis. Many clinical trials by using the inhibitors for MMP are now under way. Second are the antiangiogenic inhibitors. The target is vascular endotherial growth factors (VEGF) and their receptors. Antibodies and tyrosine kinase inhibitors against these target proteins are now being evaluated in preclinical and clinical settings. The next molecular therapeutic is an antibody against HER-2 oncoprotein. HER-2 oncoprotein was found in 1985 as a human epidermal growth factor receptor-2. HER-2 is overexpressed in various tumors including breast, ovary, lung, prostate, and colon tumors. The humanized antibody (Herceptin) against HER-2 was raised and used against HER-2 positive metastatic breast tumors (1–5). A good response was observed, and this antibody is considered for the therapy of other tumors. The forth molecular therapeutic is Gleevec against chronic myelogenous leukemia (CML). Molecular pathogenesis of CML is t (9:22) chromosome translocation, accompanying the emergence of the Philadelphia (Ph) chromosome. The produced fused protein BCR/ABL continuously transmits the growth signal into the cells, thereby forming malignant transformed tumor cells. Gleevec specifically interacts with the tyrosine-kinase domain of the BCR/ABL protein, competitively with ATP (6–9). Finally, a tyrosine-kinase inhibitor for EGF receptor has been clinically developed. The drug, Iressa, specifically interacts with tyrosne-kinase domain of EGF receptor, competitively with ATP. The target tumor is non-small cell lung cancer. Lung cancer is usually resistant to conventional chemotherapeutic agents, however, Iressa, an oral drug, showed good response (response rate, about 30% in Japan) and bears considerable interest for further development (10–14).

These drugs are evaluated as promissing molecular cancer therapeutics worldwide. In the following sections, I would like to describe our basic approaches for the development of molecular cancer therapeutics.

Multidrug Resistance

Resistance to a broad spectrum of chemotherapeutic agents in cancer cell lines and human tumors has been called multidrug resistance (MDR) (15). The MDR phenotype is associated with an increased drug efflux from the cells, which is mediated by an energy-dependent mechanism. Studies on the MDR phenotype have led to discovery of ATP-binding cassette (ABC) transporters, such as P-glycoprotein and multidrug resisitance-associated protein (MRP). Overexpression of P-gp, encoded by MDR1 gene, confers resistance to a great variety of structurally and functionally unrelated antitumor drugs such as vinblastine, vincristine, doxorubicin, daunorubicin, etoposide, teniposide paclitaxel, and many others (16). P-gp, localized on the plasma membrane of resistant cancer cells, can bind and transport the antitumor drugs in a ATP-dependent manner (Fig. 1) (17–19). The expression of P-gp has been elevated in intrinsically drug-resistant cancers of colon, kidney and adrenal gland as well as in some tumors that acquired drug resistance after chemotherapy.

Because P-gp appeared to be involved in both acquired and intrinsic MDR in human cancers, selective killing of tumor cells expressing P-gp could be very important for cancer therapy. In 1981, we reported that a calcium channel blocker verapamil inhibits active drug efflux and restored drug sensitivity in MDR cells (Fig. 1) (20). Various compounds, including calcium channel blockers and calmodulin inhibitors have been shown to enhance the cytotoxic activity of various agents (21–24). Most of the reversing agents such as verapamil, cyclosporin A, diltiazem and FK-506, are substrates for P-gp-mediated transport and competitively inhibit the transport of antitumor drugs by P-gp (25). At present, a new generation of the MDR reversal agents have been developed and are currently in clinical trials. These include non-immunosuppressive cyclosporine PSC-833 and a quinoline derivative MS-209 (24, 26). The MS-209 was shown to inhibit both P-gp and MRP.

It is important to note that any P-gp inhibitors could have deleterious effects *in vivo*, because P-gp is also expressed in normal tissues such as adrenal, gravid uterus, kidney, liver, colon and capillary endotherial cells in brain (Fig. 1) (27, 28). P-gp expressed in the normal tissues could prevent xenobiotics uptake, and therefore, many P-gp inhibitors affect the pharmacokinetics of antitumor drugs. To improve combined chemotherapy, the pharmacodynamic features of

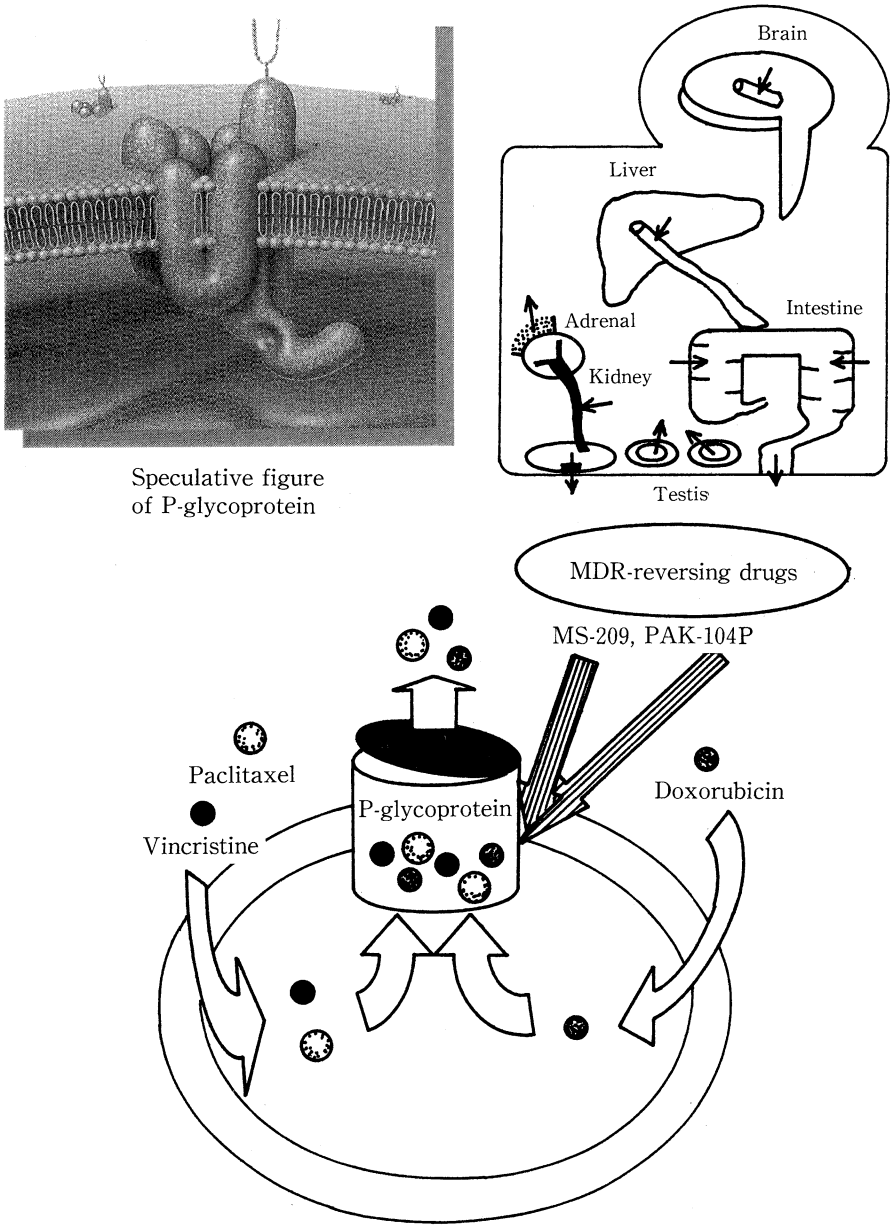

Figure 1. Function of P-glycoprotein. P-glycoprotein (speculative figure; upper left) bound to various antitumor drugs including vincristine, doxorubicine, and paclitaxel, inside the cells, and transport the antitumor drug outside the cells depending ATP energy (bottom figure). P-glycoprotein is expressed in various normal organs and function as an efflux pump of xenobiotic substances (upper right).

anti-cancer drugs and P-gp inhibitors should be carefully investigated.

Glyoxalase I as a Target in Apoptosis Resistance

Apoptosis is an active cell death mechanism that plays a role in several biological processes. Various antitumor agents have been reported to elicit apoptosis in tumor cells (29). This implies that blockade of the apoptosis signaling could be another mechanism for multidrug resistance to chemotherapy. To determine the molecular basis of resistance to antitumor agent-induced apoptosis, we isolated and characterized several drug-resistant mutants that were resistant to apoptosis induced by antitumor agents (30). To identify the factors responsible for the apoptosis resistance, we performed a cDNA subtractive hybridization with mRNA from human monocytic leukemia U937 and its variant UK711,

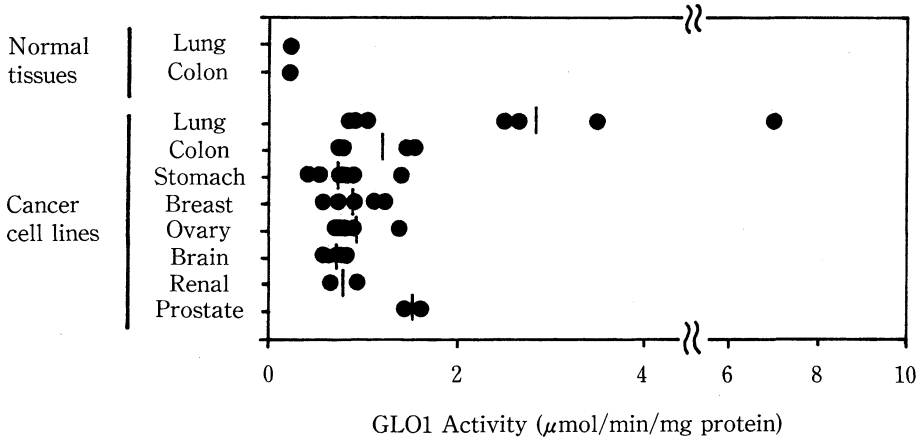

Figure 2. Glyoxalase1 enzyme activity in human solid tumor cell lines and human normal tissue samples. Cytosolic fractions were isolated, and glyoxalase1 enzyme assays were performed (4). Bars, means of each cell lines.

which is resistant to apoptosis induced by antitumor agents (31, 32). We found that glyoxalase I was selectively overexpressed in the apoptosis-resistant UK711 cells. Glyoxalase I is an essential component in pathways leading to the detoxification of methylglyoxal, a side product of glycolysis. Methylglyoxal is hypothesized to be cytotoxic to cells, owing to its protein- or DNA- modifying properties.

The mRNA expression of glyoxalase I as well as its enzyme activity was significantly elevated in several drug-resistant cells including UK711, UK110, and K562/ADM cells, as compared with their parental cells. Since these mutant cell lines were cell populations that survived after treatment with etoposide or adriamycin, it is possible that the development of drug resistance is accompanied by the overexpression of this enzyme. When overexpressed in human leukemia cells, glyoxalase I inhibited etoposide- and adriamycin-induced apoptosis, indicating the direct involvement of the enzyme in apoptosis suppression caused by these drugs. We tested the effect of the glyoxalase I inhibitors on the apoptosis resistance of human leukemia cells. We found that cotreatment with S-p-bromobenzylglutathione cyclopentyl diester (BBGC), a cell-permeable inhibitor of glyoxalase I, selectively enhanced etoposide-induced apoptosis in resistant UK711 cells but not in parental U937 cells (32). These results indicate that glyoxalase I inhibitors are effective drug resistance-reversing agents in human leukemia cells.

We quantitatively measured glyoxalase I enzyme activity in 38 human solid tumor cell lines used in our anticancer drug-screening system (32). As a result, glyoxalase I was overexpressed in lung, prostate and colon cancers, and the enzyme activity was higher in all of the cancer cell lines than in the normal tissue samples (Fig. 2). Among several cancer cells, we found that glyoxalase I was frequently and highly elevated in human lung carcinoma cells. Human lung cancer cells expressing higher glyoxalase I activity underwent apoptosis when treated with BBGC, whereas cells expressing lower activity did not (32). Moreover, the glyoxalase I inhibitor significantly inhibited the growth of xenografted human lung cancer and human prostate cancer. We observed no decrease in the body weight of glyoxalase I inhibitor-treated mice throughout the experiments. In summary, Glyoxalase I is a tumor-specific target enzyme especially in human lung carcinoma cells, and glyoxalase I inhibitors are potent chemotherapeutic agents with minimal cytotoxicity. We recently identified heat-shock protein 27 (Hsp27) as a major methylglyoxal-modified protein in cancer cells (33).

Proteasome as a Target Against Drug Resistance

Resistance to chemotherapy is a principal problem in treating most common solid tumors. Tumor cells, *in vivo*, are often exposed to such conditions as glucose deprivation, hypoxia, low pH, and other nutrient deprivation. The microenvironment itself has been thought to be a major mechanism of drug resistance because it reduces drug accessibility to tumor cells and reduces the oxygen radicals generated by antitumor drugs. In addition, the microenvironmental stress conditions may select tumor cells that have decreased apoptotic potential through genetic alterations, thereby leading to apoptosis resistance induced by antitumor drugs.

Topo II poisons stabilize the cleavable complex, an intermediate product of the topo II-catalyzed reaction. Accumulation of the cleavable complexes is thought to lead to eventual cell death, and a decrease in the number of cleavable complexes could confer drug resistance. In agreement with this, a decreased expression of topo IIa occurs under glucose-regulated stress conditions. We found that proteasome inhibition attenuated the inducible resistance by

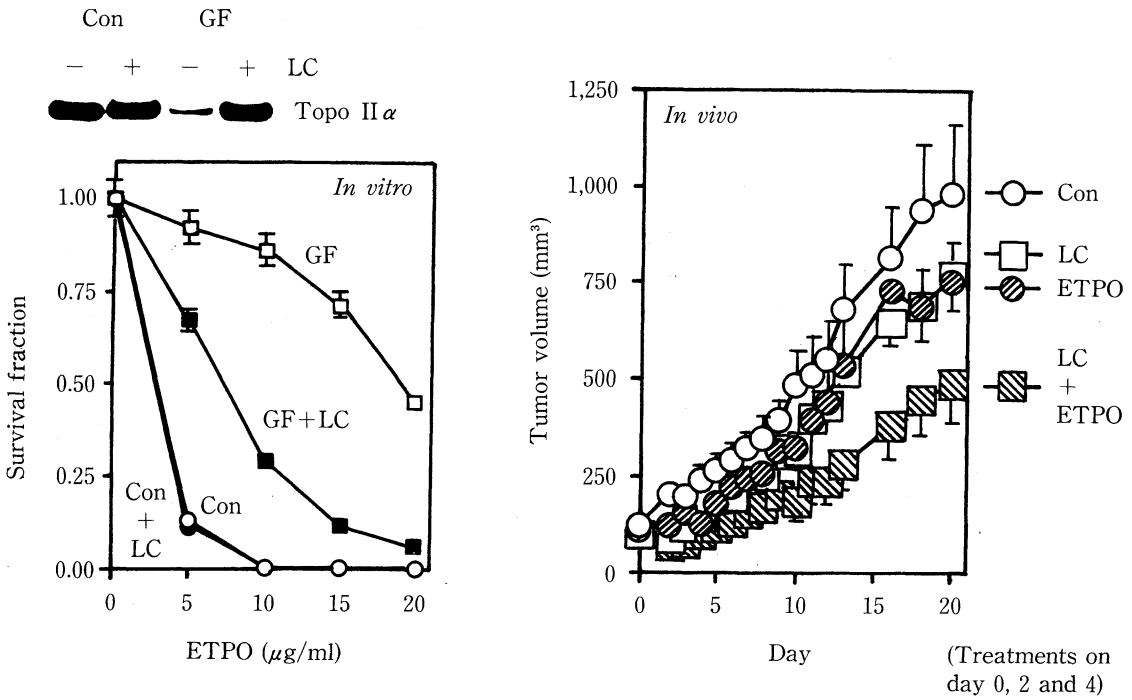

Figure 3. Inhibition of topo IIa degradation by lactacystin and enhancement of the antitumor activity of etoposide *in vitro* and *in vivo*. Experiment was carried out as described (7). Lactacystin (LC) inhabited Topo IIa degradation under stress (glucose-free; GF) conditions (upper left), there by enhancing the cytotoxicity of etoposide *in vitro* (lower left). The combination treatment with lactacystin and etoposide enhanced chemotherapeutic effect in HT-29 inoculated xenograft model (right).

inhibiting the topo IIa depletion induced by glucose starvation and hypoxia as well as by the chemical stressor A23187 (34, 35). The topo IIa restoration was seen only at the protein levels, indicating that the topo IIa protein depletion occurred through a proteasome-mediated degradation mechanism. In agreement with the topo IIa restoration, the stress-induced etoposide resistance was effectively prevented *in vitro* by the proteasome inhibitor lactacystin (Fig. 3) (35). Furthermore, lactacystin significantly enhanced the antitumor activity of etoposide in the refractory HT-29 xenograft (Fig. 3). We found that the stress conditions stimulated nuclear accumulation of proteasome in HT-29 human colon cancer cells (36, 37). Further studies into the mechanisms of nuclear proteasome transport would provide tumor-selective strategies to circumvent the inducible resistance to topo II-directed drugs.

Akt-mediated Survival-signaling Pathway as a Promising Target for Cancer Chemotherapy

The susceptibility of cells to undergo apoptosis appears to be dependent on the balance between pro-apoptotic and survival (anti-apoptotic) signals. The fact that diverse chemotherapeutic drugs induce apoptosis, while they engage different intracellular targets and cause DNA damage, raises the possibility that anticancer drugs may induce apoptosis by decreasing survival signals, such as Akt-mediated survival-signaling pathway. The serine/threonine kinase Akt (also known as PKB or RAC-PK) is the cellular homologue of the retroviral oncogene product v-Akt. Numerous reports have indicated that growth factors and cytokines stimulate cell survival. After stimulation with growth factors and cytokines, phosphatidylinositide-3-OH kinase (PI3K) is activated and phosphorylates phosphoinositides. The interaction of the generated phospholipid second messenger molecule, phosphatidylinositol 3,4,5-trisphosphate [PIP_3], with the pleckstrin homology domain of Akt recruits Akt to the plasma membrane, where it is phosphorylated (38). Phosphorylation is necessary for full activation of Akt and the subsequent control of biological responses, including apoptosis inhibition and cell cycle progression (38).

To find the molecules that interact with Akt, we performed immunoblot analysis after immunoprecipitation of Akt with an anti-Akt pAb. We could identify that Hsp90 was an Akt-binding protein (39). Examination of the role of Hsp90 in Akt signaling by inhibiting Akt-Hsp90 binding with binding domain-containing Akt deletion mutants revealed that the blockade of Akt-Hsp90 binding inactivated Akt and increased the sensitivity of the cells to apoptosis-inducing stimuli, such as growth-factor withdrawal and

chemotherapeutic drugs (39). Akt-Hsp90 binding might play an important role in the stabilization of Akt kinase activity. We recently identified that upstream Akt kinase PDK1 was a Hsp90 client protein (40). Treatment of the cells with Hsp90 inhibitors suppressed the PDK1-Hsp90 binding *in vivo* and decreased the amount of PDK1 without directly inhibiting PDK1 kinase activity. These results indicate that Hsp90 plays an important role in Akt signaling pathway by binding to both Akt and PDK1, and that Hsp90 might be a promising target for developing new chemotherapeutic drugs to suppress Akt-mediated survival-signaling pathway (41, 42). In summary, the Akt signaling pathway (particularly upstream Akt kinase PDK1) is a promising new target for developing chemotherapeutic drugs in future.

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