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Review

Glutathione transferases and development of new principles to overcome drug resistance

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ABSTRACT

Chemoresistance is a multifactorial phenomenon and many studies clearly show that a coordinated expression of efflux transporter proteins and phase II conjugating enzymes in tumor cells is linked to the development of the multidrug resistance phenotype. In particular, the overexpression of glutathione S-transferases and efflux pumps in tumors may reduce the reactivity of various anticancer drugs. In recent years it has become evident that glutathione S-transferases are also involved in the control of apoptosis through the inhibition of the JNK signaling pathway. As such, the glutathione S-transferase superfamily has become the focus of extensive pharmaceutical research in attempt to generate more efficient anticancer agents. Here we present an overview of the GST inhibitors and the GST-activated pro-drugs utilized to date to overcome drug resistance.

Multidrug resistance mechanisms

An essential target in cancer therapy is the development of novel drugs that, either alone or in combination, may suppress or evade the phenomenon known as multidrug resistance (MDR),[1] i.e. the ability of cancer cells to acquire resistance to various drugs. Chemoresistance is a multifactorial phenomenon and several mechanisms have been suggested to date.

Of these mechanisms, one of the most frequently encountered means for the acquisition of resistance by tumor cells is the induction and activation of efflux transporter proteins. The ATP-binding cassette (ABC) transport proteins, such as P-glycoprotein (Pgp), multidrug resistance associated-protein 1 (MRP1 or ABCC1) and breast cancer resistance-protein (BCRP or ABCG2), have been frequently observed to be involved in MDR [1].

Another way of obtaining MDR is through alterations in target molecules, such as mutations in topoisomerase II to gain MDR against drugs that block its activity, such as etoposide [2].

Tumor cells can also become resistant due to the enhanced repair of DNA. One example of this is cisplatin-resistant cell lines that show increased levels of DNA repair, measured through the loss of platinum adducts, DNA repair synthesis, and reactivation of cisplatin-damaged plasmid DNA [3].

Changes in genes that are critical for proliferation or apoptosis are also observed in resistant tumors; the deletion or mutation of p53 is responsible for the MDR observed in several tumor cell lines [4].

Cancer cells can also acquire resistance by overexpressing enzymes that may increase detoxification and circumvent the cytotoxic action of antitumor drugs. In particular, a number of alkylating agents, currently used in cancer therapy, are known to be substrates of glutathione S-transferases (GSTs) [5] and it has been clearly shown that overexpression of GSTs and high levels of glutathione (GSH) in tumors are linked to the development and expression of MDR [6].

The role of GST in drug detoxification

The increase of the GST levels occurs by transcriptional activation mediated by the nuclear factor-erythroid 2 p45-related factor 2 (Nrf2); under basal conditions, Nrf2 is sequestered in the cytoplasm by the Kelch-like ECH-associated-protein 1 (Keap1), thereby targeting Nrf2 for ubiquitination and proteasome degradation [7]. A frequently mentioned mechanism is that upon cell stimulation, Nrf2 dissociates from Keap1 and translocates to the nucleus inducing the expression of a set of cytoprotective genes [8]. However,

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[1] *Abbreviations used:* ARE, antioxidant response element; MDR, multidrug resistance; ABC, ATP-binding cassette; PgP, P-glycoprotein; BCRP, breast cancer resistance-protein; GSTs, glutathione S-transferases; MAPK, mitogen activated protein kinase; COMC, 2-crotonyloxymethyl-2-cycloalkenone; DIDGIC, dinitrosyl-diglutathionyl-iron complex.

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more recent data suggest that the direct disruption model for Keap1–Nrf2 is incorrect as human Keap1 and Nrf2 proteins bind with very high affinity (Kd of 9 nM). The novel model suggests that oxidative and electrophilic stresses do not alter the affinity of Keap1 for the Nrf2 but affect the Keap1-mediated Nrf2 ubiquitylation resulting in the nuclear accumulation of Nrf2 [9–11].

Once in the nucleus, Nrf2 forms a heterodimer with small Maf proteins and activates transcription of phase II enzymes via interaction with the regulatory DNA sequence termed antioxidant response element (ARE), located in the gene promoter or enhancer region [7].

The GSTs (EC 2.5.1.18) constitute a phase II multigenic family of isoenzymes that in the human are divided into seven classes: (Alpha, Mu, Pi, Omega, Theta, Zeta and microsomal) [12]. The GSTs catalyse the nucleophilic attack of the sulphur atom of GSH on the electrophilic group of numerous types of xenobiotics and could also detoxify drugs not by acting directly on the molecules but rather on a metabolite. This drastically reduces the reactivity of these compounds and makes them more water soluble so as to favour their elimination. As a result, the GST catalytic activity may play a key role in the detoxification of a variety of anticancer drugs such as chlorambucil [13,14], cyclophosphamide [5,15], melphalan [16], carmustine [17], cisplatin [18], busulfan [19], thiotepa [20], ifosfamide [21] and mitoxantrone [22,23].

The most highly expressed GST isoenzyme in various human cancerous and precancerous tissues is GSTP1-1 [24]; its expression has been associated with differences in chemotherapeutic response and cancer susceptibility to anticancer drugs such as cisplatin [25] and chlorambucil [14] and inhibition of Pi-class GST expression, through antisense cDNA, has been shown to increase the tumor sensitivity to adriamicin, cisplatin, melphalan and etoposide [26]. Overexpression of the Alpha, Mu, microsomal and Theta-class GSTs may also provide protection to cancer cells. In fact, overexpression of Alpha class GST has been correlated with the resistance to alkylating agents [27] and to doxorubicin [28,29]. Overexpression of Mu class GST has been associated with chlorambucil resistance in human ovarian carcinoma cell line [13] and with poor prognosis in childhood acute lymphoblastic leukaemia [30]. Moreover, patients with breast cancer, presenting GST Mu (GSTM1) and Theta (GSTT1)-null genotypes have been shown to have a reduced hazard of death in relation to those with alleles present [31]. The membrane-bound microsomal GST (MGST1) has been reported to protect cells from chlorambucil, melphalan and cisplatin [32] and recently, overexpression of MGST1 in primary tumors from patients with localized Ewing sarcoma has been associated with poor prognosis for doxorubicin treatment [33].

It is noteworthy that overexpression in tumor cells of the GSTs may be responsible for the resistance towards drugs such as doxorubicin and etoposide, which are not known substrates for GSTs [6,34,35]. This phenomenon may be explained by invoking another mechanism described in the following section.

The GST and its non catalytic role

Besides its active role in the detoxification of antitumor drugs, in recent years a great deal of evidence has shown that GSTs, and in particular GSTM1-1 and GSTP1-1, are also involved in the regulation of apoptosis through the inhibition of the c-Jun-N-terminal kinase (JNK) signaling pathway [12].

JNK is activated by multiple diverse stimuli leading to varied and seemingly contradictory cellular responses. JNK has a role in enhancing cell survival and proliferation, yet its activation may also result in apoptosis and is required for the cytotoxic effect of a variety of chemotherapeutic agents [36].

Adler et al. demonstrated that elevated levels of GSTP1-1 sequester and inhibit the activity of JNK and protect tumor cells from apoptosis [37,38]. This explains why GST-mediated MDR is observed with anticancer drugs that are not substrates of GSTs but require the mitogen activated protein kinase (MAPK) pathway activation to induce apoptosis [6,39].

Wu and colleagues [40] have shown that GSTP1-1 is also physically associated with TNF Receptor Associated Factor 2 (TRAF2) in human cervical carcinoma HeLa cells. Specifically, TRAF2 is an adaptor protein which mediates the signal transduction of different receptors and is required for the activation of the apoptosis signal-regulating kinase (ASK1) [41], which in turn activates both MKK4/7-JNK and MKK3/4/6-p38 signalling pathways, both of which are known mediators of cell response to stress factors [42].

Overall these findings show that GSTP1-1 plays an important regulatory role in both intrinsic and extrinsic signaling pathways and explain why the elevated expression of GSTP1-1 has been implicated in the resistance to apoptosis initiated by a variety of stimuli. It has also been noted that GSTM1-1, a GST belonging to the Mu class, interacts with the N-terminal portion of ASK1, inhibiting its activity. It has been shown that a thermal shock, resulting in the dissociation of the GSTM1-1/ASK1 complex, enables the triggering of ASK1 and the consequent phosphorylation of JNK and p38 [43].

The aforementioned observations illustrate how the hyperexpression of GSTs in cancer cells represents a protection mechanism for the latter against stress caused by anticancer drugs. As such, cancer cells can acquire resistance to these drugs and may no longer respond to apoptotic stimuli.

The GSTs and the efflux pumps

Efflux pumps are often over-expressed in various cancers and mediate the extrusion of a wide range of therapeutic agents used for tumor treatment. Various studies have proven that there is a synergistic interaction between phase II enzymes and efflux pumps in conferring resistance towards multiple anticancer drugs [44]. Moreover, the coordinated expression of efflux transporter proteins, GSTs, and γ-glutamylcysteine synthetase (γ-GCS) (the rate-limiting enzyme in glutathione synthesis) provides a more efficient protective phenotype and is frequently observed in drug-resistant cells. An increased expression and activation of these proteins leads to a decrease in drug accumulation by the cell and allows tumor cells to obtain resistance. The most common of the efflux transporters are the ABC transporters Pgp, MRPs and ABCG2 (also known as mitoxantrone-resistance protein) [45]. Beyond the considerable functional redundancy between these transporters, the substrate selectivity of the pumps differs markedly. In particular, the MRPs are involved in the transport of GSH, glucuronate or sulphate conjugates of organic anions that arise from detoxification reactions by phase II conjugating enzymes such as GSTs, sulfotransferases and UDP-glucuronosyltransferases (UGT). MRPs also export GSH and the co-transport with GSH is required for the MRP mediated extrusion of oncolytic drugs [46].

There are a number of examples in the literature of a synergism between MRPs and the most representative human GST isoenzymes (see Table 1); a synergism between these pumps and the GSTA1-1 has been observed in the resistance to chlorambucil. The detoxification of this drug by GSTA1-1 requires the removal of the glutathione conjugate by either MRP1 [47] or MRP2 [48] transporters. GSTM1-1 can act in synergy with MRP1 to protect melanoma cells from toxic effects of vincristine [49] and coexpression of GSTP1-1 with MRP1 confers significant resistance to chlorambucil, vincristine, ethacrynic acid, and etoposide [44,50].

The cis-acting regulatory elements, ARE, play a central role in the concerted expression regulation of phase II enzymes and MRPs. Evidence of the role of Nrf2–ARE in the expression of phase II enzymes and MRPs was mainly provided in studies using Nrf2 knock-