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The role of GSTπ isoform in the cells signalling and anticancer therapy

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Abstract. – OBJECTIVE: The glutathione S-transferases (GSTs) overexpression in hyperproliferating tumour cells resistant to chemotherapy was demonstrated. An increased GST-π activity weakens the efficacy of anti-cancer drugs by promoting their efflux from cells.

MATERIALS AND METHODS: This review summarises available information on the physiological role of GSTs, in particular the role of GST-π, in regulation of signalling pathways mechanisms and cellular homeostasis for understanding and explaining the basis for GST-π application as a target for anticancer therapy and implications for clinical practice.

RESULTS: GST-π can weaken the effect of TNF receptor-associated factor 2 (TRAF2) on apoptosis signal-regulating kinase-1 by inactivation of MAP kinase pathways (c-Jun N-terminal, p38 kinases). GST-π is involved in the metabolism of endogenous lipids mediators, such as 15-detoxy-$\Delta^{12,14}$-prostaglandin J2 (15d-PGJ2). Reduced binding of 15d-PGJ2 to peroxisome proliferator-activated receptors accompanied by GST-π can result in the inhibition of apoptosis. GSTP1 RNA is able to increase the phosphorylation of signal transducer and activator of transcription 3, what results in negative regulation as regards transcriptional activity thereof and affects the growth factor signalling. However, the oxidation of GST-π results in inhibition of TRAF2-GST-π complexes formation and unblocks cell apoptosis. The inhibition of multidrug resistance related proteins 1 (MRP-1) promoter activity and impairment of MRP-1 function can also act as a potent non-competitive inhibitor of GST-π.

CONCLUSIONS: GST-π is recognised as an important target in designing new anticancer drugs. These drugs are often substrates for GST-π or have the affinity with its structure, what results in weakening its activity and achieving therapeutic goal.

Key Words:

Structure of glutathione S-transferase isoforms, Catalytic activity, Reduced glutathione, Reactive Oxygen Species (ROS) inactivation, S-glutathionylation of drugs, Anticancer therapy, Signalling pathways.

Abbreviations

15d-PGJ2 = 15-detoxy-$\Delta^{12,14}$-prostaglandin J2; AP-1 = activator protein 1; ARE = antioxidant response elements; ASK1 = apoptosis signal-regulating kinase-1; ATP = adenosine-5'-triphosphate; CPIC = p-chlorophenyl isocyanate; GSH = reduced glutathione; GST = glutathione S-transferase; GSTπ = π izoform of glutathione S-transferase; JNK = c-Jun N-terminal kinases; Keap1 = Kelchlike ECH-associated protein 1; MAPK = mitogen-activated protein kinases; MRP = multidrug resistance related proteins; NBDHEX = 6-(7-nitro-2,1,3-benzoxadiazol-4-ylthio) hexanol; NFκB = nuclear factor kappa-light-chain-enhancer of activated B cells; Nrf2 = nuclear factor erythroid 2-related factor 2; PAO = phenylarsine oxide; PPARs = peroxisome proliferator-activated receptors; Prdx6 = peroxiredoxin-6; SN-38 = irinotecan hydrochloride; STAT3 = signal transducer and activator of transcription 3; TLK199 = ezatiostat hydrochloride; TNF = tumour necrosis factor; TRAF2 = TNF receptor-associated factor 2.

Introduction

Glutathione S-transferase (GST, EC 2.5.1.18) is a superfamily of protein enzymes widely distributed in nature — from bacteria to animals[1,2]. So far, seven classes of GST have been detected and described in the mammalian cytoplasm, namely: GSTA (alfa;α), GSTM (mu;μ), GSTP (pi;π), GSTS (sigma;σ), GSTT (theta;θ), GSTZ (zeta;ζ), GSTO (omega;ω)[3-5]. GST isoforms present in the cellular mitochondria, nucleus, peroxisomes, cell membrane are also known[4,6]. It is proven that the microsomal GSTs, also named as membrane-associated proteins, that are involved in eicosanoid and glutathione metabolism (MAPEG), constitute integral membrane proteins which are not evolutionary related to the other major classes[7].

The GST gene family is encoded by genes grouped into chromosome clusters[2]. Five genes

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coding isoenzymes of GST-α (GSTA 1-5) and GST-μ (GSTM 1-5) classes were defined, which are located in chromosome 6 and 1, respectively[8]. The GST-θ class is coded by three genes named GSTT1, GSTT2 (gene/pseudogen) and GSTT2B (gene/pseudogen), which are located on chromosome 22[8]. GST-σ, GST-ζ and GST-π classes are coded by a single gene located on chromosomes 9, 14 and 11, respectively[2-4,6,9,10], while the GST-ω class – by two genes (GSTO1 and GSTO2) located on chromosome 10[5].

The primary function of GST is to catalyse the coupling reaction of reduced glutathione (γ-Glu-Cys-Gly, GSH) with electrophilic carbon, sulphur or nitrogen atoms to create many non-polar and hydrophobic organic compounds, including carcinogens, chemotherapeutics and environmental xenobiotics[3,6,8,9,11]. GST has the ability to deprotonise and reduce GSH that lowers the pKa value of its thiol group[9,12]. This process allows to form a highly nucleophilic thiolate anion (GS-) under physiological conditions, as well as thioether bonds between enzyme and detoxified molecules[4,9,13]. Hydrophilic GSH conjugates, generated in this way, can be removed from the cell by membrane-bound transport proteins (pumps) belonging to the C protein subfamily binding adenosine-5'-triphosphate (ATP), such as multidrug resistance related proteins (MRP/ABCC)[14]. In the next stage of this reaction, glutamine and glycine residues are separated from GSH. The conjugates formed with cysteine can be returned to the cell, where they are mostly acetylated to form mercapturic acid derivatives. The mercapturic acids formation accompanied by GST, is the first step for a physiologically important process allowing to excrete endogenous and exogenous electrophilic compounds in bile or urine[3,7,14-16]. However, the above-mentioned process does not always result in the inactivation or reduction of harmful compounds toxicity. It has been shown that as an increase in toxicity of short-chain alkyl halides may be the result of reaction catalysed by GST. After their enzymatic binding to GSH, these compounds can bind to DNA and induce changes in gene expression[15]. Additionally, GSH activated as a thiolate anion, can bind SO-tioperoxols (-SOH) in proteins, which contain cysteine residues with low pKa in their structures[13,16].

Except the ability to catalyse coupling of hydrophobic or electrophilic compounds to endogenous glutathione, GST performs many different biological functions. It was reported that some of GST isoforms may protect cells against oxidative and nitrosative stress, and may be involved in synthesis or modification of leukotrienes and prostaglandins[7,17]. Some of them are characterised by the activity of glutathione peroxidase, thiol transferase and even isomerase[7,18]. Furthermore, GST performs chaperones functions[4] and have the ability, like ligandins, to bind non-substrates compounds, such as heme, bilirubin, steroids, free fatty acids, thyroid hormones[19]. GST is involved in regulation of nitric oxide (NO) pathways[4]. In addition, some GST isoforms may perform non-enzymatic functions by regulating cellular signalling pathways[3,7,9].

In many studies, it was observed that the GST family members, in particular GST-π, are expressed abundantly in tumour cells and can promote carcinogenesis[20-25]. This review summarises available information on the physiological role of GSTs, in particular the role of GST-π, in regulation of signalling pathways mechanisms and cellular homeostasis for understanding and explaining the basis for GST-π application as a target for anticancer therapy and implications for clinical practice.

The Structure of GST Isoforms

The structure of each GST isoform is formed by a single protein chain composed of 190-244 amino acids[6]. In the structure of cytosolic GSTs, two following domains were distinguished: N-terminal domain I and C-terminal domain II, which are bounded together by a short α-helix with variable length[15]. The I domain consists of both α-helixes and β-folding structures. It comprises the initial amino acid positions in the polypeptide chain[18,26]. Further positions of polypeptide chain form α-helixes, which number varies, depending on GST class, and they are characteristic of II domain[26]. The N-terminal domain I has a fairly constant structure, while the C-terminal domain II is highly variable among GST cytosol isoforms[18].

It has been shown that each of mammalian cytosolic GST may be in the form of a dimer composed of two polypeptide chains constituting two subunits of enzyme[18]. Additionally, depending on the number of genes coding the polypeptide chains of GST, the isoenzymes of the same classes can exist as homodimers or as heterodimers. The appearance of heterodimeric forms of this enzyme within GST-α and GST-μ classes was demonstrated[6,18,19,27].

The balance between monomers and dimeric form of the enzyme is influenced by electrostatic

interactions of amino acid residues included in various subunits[28]. In the crystal structure of GST, in its dimeric form, two main areas of interaction of dimer building subunits were shown: the hydrophobic and electrostatic region[28]. In GSTs, a typical hydrophobic contact, which is important for inter-subunit communication, constitutes "lock and key" motif[29]. It was shown that this region is formed by hydrophobic pocket in C-terminal domain of one subunit (lock apparatus) and aromatic residue (key residue) in N-terminal domain of the other subunit[27]. This motif ensures conformation of the active centre and is a common feature of GST-α, GST-μ and GST -π classes[27]. In the above mentioned classes of GST, a key residue is phenylalanine or tyrosine (Tyr-49 or Tyr-50 with Met-1 residue in hGSTP1-1) incorporated in ahydrophobic pocket formed by Met-91, Val-92, Gly-95, Pro-128, Phe-129, and Leu-132 of the second subunit chain[27].

It was demonstrated that the "lock and key" region is also included in the structure of GST-ζ and GST-θ classes. However, with regard to these classes, it was shown that the structure of the said motif is different than in GST-α, GST-μ and GST -π classes[27]. Additionally, the pi-pi aromatic interaction between the rest of keys of two subunits was shown in the structure of GST-ζ and GST-θ classes. This region of enzyme was named the "buckle" motif[27]. Similar motifs were not detected between subunits in GST-ω class[27].

In each subunit of GST it is possible to distinguish place G and H, which form the active centre of enzyme (Figure 1)[15,30,31]. It has been proven that place H adheres to place G in the gap between domains of GST subunit[6,32]. The area called place G is located in the hydrophilic gap between the domains of subunit[18,26]. The place G is characteristic of binding reduced glutathione, i.e. a physiological substrate giving GST[18,7]. It has been shown that G site in GST-α, GST-μ and GST-π classes is more easily available for GSH compared to GST-θ and GST-ζ classes[15]. However, place H is formed by domain II and a loop from domain I[19,26,28]. Conformation of this site allows to bind various electrophilic substrates to enzyme[30]. It has been proven that different amino acid sequences at H site can alter the substrate specificity of GST isoenzymes[6,32].

Depending on GST class, a different catalytically significant amino acid residues in the active centre of enzyme were distinguished. In was shown that the catalytic activity of enzyme is conditioned by presence of a tyrosine residue in the case of GST-α, GST-μ, GST-π, GST-σ, serine residue – in GST-θ and GST-ζ and cysteine residue in active site of GST-ω[15,18]. These amino acid residues have the ability to react directly with GSH thiol group[9,18,33].

It was demonstrated that particular amino acid residues determining the catalytic activity of enzyme are located in different sites within GST structure. The tyrosine residue is located in I domain, in the first β-structure of protein chain, while serine and cysteine residues are located in the first α-helix[34]. Therefore, the division of cytosolic GST into two subgroups was applied. The first of GST subgroups was defined as a tyrosine type (Y) and included GST-α, GST-μ, GST-π and GST-σ classes. Despite of minor differences in the structure, such as additional α-helix in the active site of GST-α or hydrophilic contact area in GST-σ subunits, it was believed that these classes are very similar to each other within the cytosolic GST family[34,35]. The other subgroup comprises GST-θ, GST-ζ and GST-ω classes, containing serine or cysteine residues in the active centre (S/C GST)[9,34]. It was shown that hydroxyl groups of tyrosine and serine residues that are present in the active centre of enzyme, can form hydrogen bonds with the sulphur atom in GSH molecule[18]. This process can lead to enzyme activation. However, the presence of catalytic cysteine residue in GST-ω polypeptide chain, enables formation of disulphide bond with GSH, whereby the active

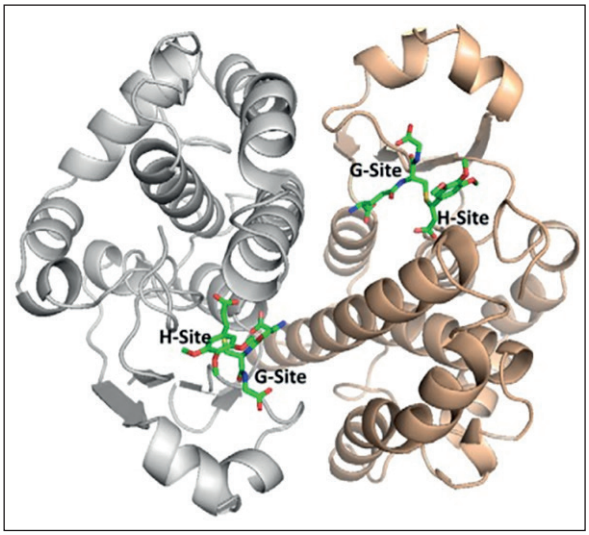

Figure 1. The structure of GST-π dimer[31]. A subunit is coloured in brown, B subunit is grey. In each subunit the glutathione (G-site) and hydrophobic (H-site) binding sites are labelled. The site for the formation of xenobiotics-GSH complex is coloured in green.

centre of enzyme becomes inaccessible to the coupling reaction[32,34]. It was shown that the active centre containing catalytic tyrosine residue is more easily available for GSH compared to isoenzymes with other amino acids residues[15].

The Importance of GST-π in the Regulation of Cells Signalling Pathways

GST-π (known as GSTP1-1; GSTP; GST-pi; GST3; DFN7; FAEES3; HEL-S-22) is coded by a gene including six introns and seven exons, located on the 11th chromosome (11q13.2)[3,4,9,10] GSTP1-1 isoenzyme is the only representative of GST-π class in humans. GST-π is the most common isoform of glutathione transferases present in erythrocytes (e-GST)[36,37]. The π isoform is also widespread in respiratory tracts, lungs, skin, brain and heart[3,19]. Initially, its presence was confirmed only in cytoplasm, nevertheless, later on it was also detected in the mitochondria of liver cells. In tumour cells, π isoform was also located in the cell nucleus and lysosomes[4,38,39]. It was reported that intracellular GST-π exists as a dimer with molecular mass of 46-47 kDa[6,28,40] being in reversible balance with the monomeric subunits[28,41]. The wild type π isoform can form heterodimers with other isoenzymes and proteins outside this superfamily.

The analysis of GST-π class structure showed four cysteine residues at positions 14, 47, 101 and 169 of the polypeptide chain in each subunit[42]. Due to the presence of thiol groups in its structure derived from cysteine residues, this enzyme is extremely sensitive to changes in the redox potential in the cell[4]. It was shown that oxidative stress can strongly induce the expression of GST isoenzymes by binding transcription factors such as Kelchlike ECH-associated protein 1 (Keap1), nuclear factor erythroid 2-related factor 2 (Nrf2) and activator protein 1 (AP-1) to antioxidant response elements (ARE) in their gene promoters[9,16,43,44]. The complex of Keap1/Nrf2/ARE is responsible for maintaining redox homeostasis in physiological or stress conditions. Genes regulated via ARE are involved in gene transcription for antioxidants synthesis, including detoxifying enzymes of the II phase (e.g. GST) and maintaining GSH homeostasis. However, it is possible to form a disulphide bridge between highly reactive cysteines in GST molecule under oxidative stress conditions. In the case of π isoform, cysteine residues at positions 47 and 101 of the polypeptide chain are involved in this process[3,19,42]. The formation of disulphide bridge in GST-π molecule can result in oligomerization and complete inactivation of the enzyme[16,19]. It was confirmed that the oxidised form of this enzyme was present in human saliva but was not found in the blood of mammals[3,42].

The specific oligomerization of the enzyme, leading to its irreversible inactivation, can also occur under the influence of 15-detoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2), i.e. an endogenous lipids mediator, in which metabolism the GST-π is engaged[45]. 15d-PGJ_2 is able to posttranslational alkylation of the cysteine residues in positions 47 and 101 of GST-π polypeptide chain, what can result in conformational changes and enzyme inactivation[46,47]. On the other hand, it was shown that 15d-PGJ_2 is able to induce GST-π through binding of various proteins, including c-jun, to responsive element present in GSTP1 5'-flanking region[47].

It was provided that the 15d-PGJ2 activity is important in the regulation of transcription factors by affecting Nrf2 factor[48]. 15d-PGJ2 is capable to modify the cysteine residues in Keap1 binding Nrf2[48]. This process allows the Nrf2 protein to avoid binding by Keap1 and inhibit the ubiquitination process (proteasomal degradation). As a result of the aforementioned process, Nrf2 can be accumulated in the cell nucleus, where it induces the expression of ARE-dependent genes[9,16,44] (Figure 2). It was reported, that as the effect of it, the cell may increase resistance to oxidative stress, including, but not limited to, by increasing GSH synthesis and GST induction , as well as MRP pump[9,44]. In turn, the conjugation of 15d-PGJ2 and GSH catalysed by GST, abolishes the ability to modify Keap1, and therefore it induces a decrease in the expression of antioxidant response genes.

It was shown that 15d-PGJ_2 can inhibit the signalling pathways induced by the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). This process is targeted on IκB kinase (IKK) by a covalent binding on Cys-179, or by direct inhibition of binding of NF-κB to DNA specific sequences by modifying Cys-38 in NF-κB 1. It reduces the release of proinflammatory cytokines, i.e. among others tumour necrosis factor α (TNF-α) and interleukin 6 (IL-6)[48] (Figure 2). The scientific reports also showed that the abolition of GST-π activity improved transport of NF-kB factor to the cell nucleus, its transcriptional activity and increased production of proinflammatory cytokines[49,50]. On the other hand, data from *in vitro* studies indicated that stimulation with lipopolysaccharide (LPS) leads to a transient increase in π isoform activity in the cell nucleus[49]. It was shown that increased GST-π activity may

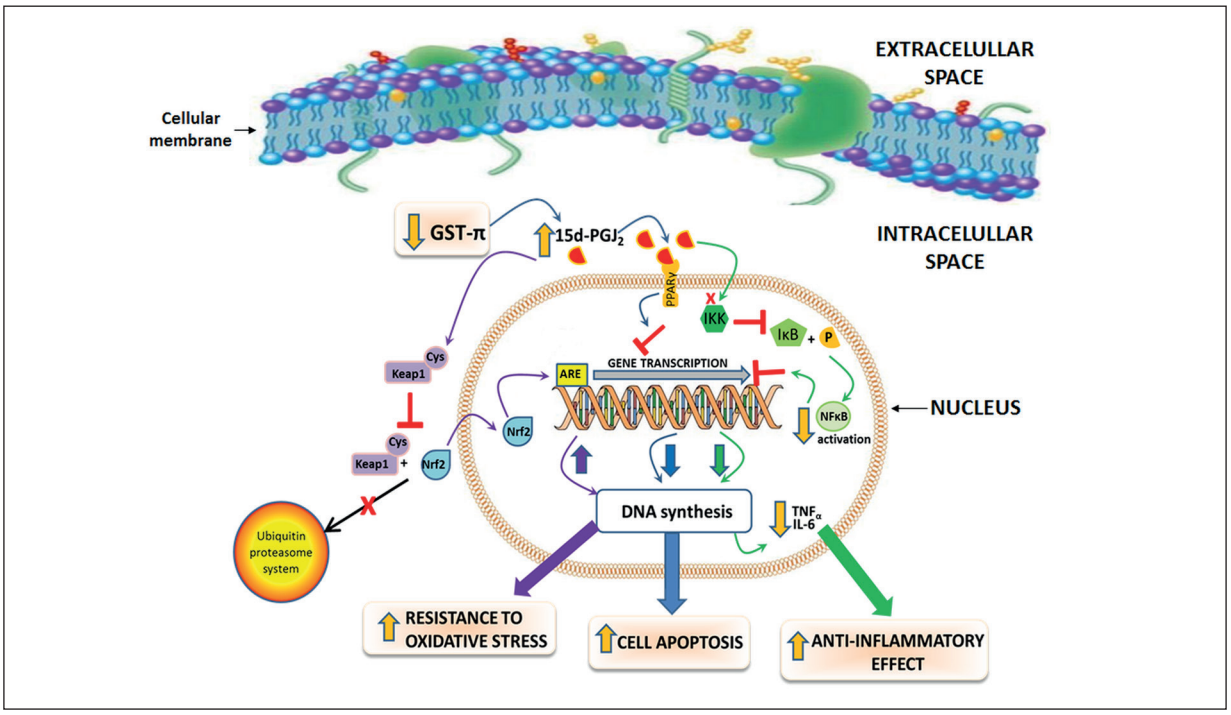

Figure 2. Molecular effects of decreased GST-π activity in the cell. A decreased GST-π activity can enhance the concentration of 15d-PGJ2 and its ability to PPARγ activation. The 15d-PGJ2-PPARγ complex formation results in the inhibition of gene transcription, decreased DNA synthesis and orientation of cellular pathways to apoptosis. An increased 15d-PGJ2 concentration causes the inhibition of IκB phosphorylation through IKK inactivation. As a result of this process, a decrease in NFκB activity and limitation of its binding to DNA is observed, what contributes to decreased cytokines production and causes anti-inflammatory effect. 15d-PGJ2 is able to modify cysteine residues in Keap1 thus preventing Keap1-Nrf2 complex formation, which makes possible to avoid its proteasome degradation. Free Nrf2 undergoes translocation to the nucleus, where it induces activation of ARE-dependent genes transcription and increase in antioxidants synthesis. 15d-PGJ2: 15-detoxy-$\Delta^{12,14}$-prostaglandin J2; ARE: antioxidant response elements; GSTπ: π izoform of glutathione S-transferase, IKK: IκB kinase, IL-6: interleukin 6; IκB: inhibitor of κB; Keap1: Kelchlike ECH-associated protein 1; NFκB: nuclear factor kappa-light-chain-enhancer of activated B cells; Nrf2: nuclear factor erythroid 2-related factor 2; P: inorganic phosphor, PPARγ: peroxisome proliferator-activated receptors γ; TNFα – tumor necrosis factor α.

inhibit LPS-induced efflux of the high mobility group box protein 1 from the macrophage cell nucleus, that can play the role of proinflammatory cytokines in extracellular space[49].

The studies conducted on animals have shown that overexpression of GST-π can reduce 15d-PGJ2 capacity to activate nuclear peroxisome proliferator-activated receptors (PPARs)[16]. PPARs are involved in glucose and lipid metabolism, energy balance, as well as in regulation of growth, cell cycle and differentiation of hepatic oval cells[51,52]. As demonstrated in earlier studies, binding of 15d-PGJ2 to PPARγ in mouse liver resulted in the induction of hepatocyte growth factor (HGF) thus causing an increase of apoptosis and a decrease in DNA synthesis in HepG2 cells[53]. Therefore, it can be assumed that reduced binding of 15d-PGJ2 to PPAR in which GST-π is involved, can result in the inhibition of apoptosis through the above mentioned mechanism. Conversely, the inhibition of GST activity can enhance formation of 15d-PGJ_2-PPARγ complex, thus resulting in the inhibition of gene transcription and activation of cellular pathways leading to apoptosis (Figure 2).

It was shown that GST-π class performs regulatory functions of signalling pathways by reacting with proteins such as mitogen-activated protein kinases (MAPK), in particular c-Jun N-terminal kinases (JNK), TNF receptor-associated factor 2 (TRAF2), signal transducer and activator of transcription 3 (STAT3) or peroxiredoxin-6 (Prdx6)[7,19,54-56].

An inhibitory effect of GST-π on TRAF2, especially in G_0/G_1 phase of non-stimulated cells was demonstrated[4,7]. According to the studies carried out on cell lines and *in vivo*, it has been shown that high concentrations of π isoform re-

sult in the formation of complexes with this factor on non-enzymatic basis. Interestingly, data analysis suggests, that only one of GST-π monomers is involved in this reaction, while the other may still perform catalytic functions[7]. Many studies reported that GST-π can also weaken the effect of TRAF2 on apoptosis signal-regulating kinase-1 (ASK1) and inhibit the effects of TRAF2-ASK1, which block the apoptosis target by inactivating MAP kinase pathways, such as JNK kinase and p38 kinase[4,19,41,54,57,58].

GST-π also has the highest ability to maintain low JNK kinase activity (among other classes, such as GST-μ and GST-α) by binding them directly to complexes[4,19]. This isoenzyme is also capable of direct binding of p38 kinase belonging to MAPK group[56]. According to the studies conducted on human umbilical vein endothelial cells, (Huvec[3]), it has been shown that GST-π class has additional ability to inactivate the p38 kinase signalling pathway (p38/MAPK/HSP27), thereby limiting cellular apoptosis[56]. This process also leads to reduction of excessive permeability of endothelial cells and thus, it has anti-inflammatory effect[56]. On the other hand, it has been proven that GST-π can increase cellular signals induced by Fas protein, which lead to apoptosis in epithelial cells[4]. Thus, GST-π, by affecting activation of kinases, can regulate the enzymatic pathways affecting cellular longevity or programmed death[19,41].

It was shown that JNK kinase dissociation from the complex formed with GST-π, results in phosphorylation of many nuclear substrates, including transcription factors constituting the active AP-1 transcription complex. It can influence the changes in expression of many genes involved in the growth process control, cellular longevity/apoptosis, inflammatory response and repair of DNA damage[4,19,41]. In this way, GST-π can act as a regulator of intracellular changes with disturbed oxidation and reduction potential[4].

The studies conducted on human hepatocellular carcinoma have demonstrated that GST-π plays an important role in negative regulation of STAT3 transcriptional activity and affects epidermal growth factor signalling[55]. The transient expression of GSTP1 specifically downregulated epidermal growth factor (EGF)-mediated tyrosine phosphorylation of STAT3, and subsequently suppressed transcriptional activity of STAT3. By contrast, GSTP1 RNA was able to cause an increase in phosphorylation of STAT3[55]. Thus, GST-π can be a regulator of the cell growth via EGF signalling.

GST-π has thiolase activity and has been identified as the major isoenzyme of glutathione transferases catalysing reversible protein S-glutathionylation[4,13,15,38,57]. This process is favoured as a result of events related to endogenous, oxidative or nitrosative stress, induced by exposure to drugs or exogenous substances[26]. S-glutathionylation involves post-translational modification of proteins, which results in binding of GSH to exposed protein cysteine residues[33]. This process protects the cysteine residue against irreversible oxidation, allowing regeneration to its reduced form during subsequent deglutathionylation[16,38]. S-glutathionylation allows to modulate the functions of many proteins, including the enzyme containing thiol group in the active centre. These are the following proteins: cytoskeleton, proteins associated with energy conversion and signalling molecules or transcription factors[26]. *In vivo* studies have shown that GST-π isoenzyme may increase S-glutathionylation and, at the same time, inactivation of the above mentioned Keap1 protein[43]. Through its thiolase activity, GST-π also mediates S-glutathionylation of apoptosis antygen 1 (APO-1) and takes part in regeneration of cysteine in the active site of Prdx6 – a member of peroxiredoxin family of antioxidant enzymes[4,16,57]. Under oxidative stress condition, SO-thioperoxol with a catalytic cysteine residue leading to inactivation of Prdx6 may be formed[4]. It has been shown that GST-π may provide a reducing equivalent in GS^- form, derived from GSH molecule, to the cysteine residue modified at position 47 of Prdx6 polypeptide chain. This results in transient S-glutathionylation followed by formation of disulphide bond between cysteine residue at position 47 of Prdx6 polypeptide chain and cysteine residue at position 47 of GST-π polypeptide chain, thus resulting in heterodimerization of these enzymes. Therefore, it becomes possible to reduce catalytic cysteine residues by means of GSH, dissociation of heterodimer and regeneration of the active form of Prdx[4].

The Role of GST-π in Anticancer Therapy

GST-π has the ability to modulate cellular signals determining cell growth, differentiation or apoptosis[59]. Therefore, this enzyme can act as inhibitor to prevent tumour cell apoptosis. Additionally, this enzyme can reduce the oxidative stress-induced formation of exocyclic DNA products. These processes can enhance the cell longevity[60].

In many studies were observed the overexpression or an increased enzyme activity in hyperproliferating tumour cells resistant to chemotherapy drugs[22-24,61]. On the basis of the immunohistochemically staining of breast cancer cells was showing that the chemotherapy was not effective in patients with GST-π (+) expression. However, in the patients with GST-π (-) expression, the applied treatment significantly inhibited tumour growth, cell proliferation and induced apoptosis cases[62]. This fact can be explained by incorrect regulation of kinase pathways in excessively proliferating cancer cells, what can result in maintenance of cell homeostasis by increasing expression of GST[26]. An increased GST-π activity weakens drugs efficacy by promoting their efflux from cells. Therefore, GST-π isoenzyme and its overexpression (at the level of protein and transcript) in tumour lines has become a potential goal in therapy.

It was shown that there is a connection between GST-π activity and cancer cells resistance to anticancer drugs and chemical compounds, even those that are not known as substrates of this enzyme[4,14,19,33,41,57]. Due to the fact that this isoenzyme has a relatively low affinity to most anticancer drugs compared to other GST classes, drug resistance may be a result of signalling pathways regulation induced by GST-π and affecting cell longevity[19,33]. It mainly concerns cancer cells of the colon, stomach, pancreas, lungs, kidneys, cervix, breasts and melanoma or lymphomas[15,19,26,39]. It was shown that oxidation of GST-π isoenzyme resulted in inhibition of formation of TRAF2-GST-π complexes which unblocks cell apoptosis[63]. Additionally, it was demonstrated that inhibitors of the nuclear transfer of GST-π complexes have practical value in promoting an increase of sensitivity to chemotherapy drugs, such as cisplatin (CDDP: Tokyo, Japan), doxorubicin (DOX: Osaka, Japan) and irinotecan hydrochloride (CPT-11: Tokyo, Japan)[64]. The metabolism of the above mentioned drugs involves GST-π, nevertheless, it was shown that GSTs may present different affinity for different drugs and their metabolites[65]. Cisplatin (a terminal half-life: 20-30 hours) undergoes the hydrolysis spontaneously to aquated species that can bind DNA and cause toxicity or that can be conjugated with GSH by glutathione S-transferases[66]. This drug is actively transported into proximal tubules of kidneys, and in the form of cisplatin-GSH complex, can be cleaved by γ-glutamyltranspeptidase and aminodipeptidase to cisplatin-cysteine, and then metabolised by cysteine S-conjugate β-lyase to form a reactive thiol. The aforementioned process is the basis of nephrotoxic effect of cisplatin. It is known that cisplatin-GSH complex can be efflux from the cells with participation of MRP, what can result not only in drug toxicity but also in cell cisplatin resistance[66]. The studies conducted on human embryonic kidney cells have shown that the overexpression of human MRP increased cisplatin resistance by 10-fold[67]. However, doxorubicin - a quinone-containing drug (a terminal half-life: 30-90 hours), can be reduced to a semiquinone, which in the presence of molecular oxygen, generates superoxide, as the semiquinone is converted to quinine. The one electron reduction of doxorubicin augments both its tumour toxicity and its toxicity towards the host, in particular its cardiotoxicity. In contrast, the two electron reduction (hydroxylation) of doxorubicin, strongly reduces its ability to kill tumour cells, while augmenting cardiotoxicity through accumulation of these cells within cardiomyocytes and their direct effects on excitation/contraction coupling within the myocytes[68]. Doxorubicin reduction results in formation of significant amount of ROS that mediate protein oxidation, lipid peroxidation, as well as base propenals formation, in which detoxification GSTs are involved[65]. GSTs may contribute to doxorubicin resistance by catalysing the detoxification of two types of toxic lipid peroxidation products: lipid hydroperoxides and hydroxyalkenals with the application of GSH[69]. GSH conjugates containing these endogenous substances, stimulates MRP-mediated transport of drug. It can confirm that GSH-GST system guarantees resistance to certain antineoplastic agents[69].

Similar mechanism of chemoresistance induction was observed in the case of irinotecan hydrochloride (a terminal half-life: 10-20 hours). It is known that this drug can contribute to increased level of lipid peroxidation products in cell nucleus, and the nuclear GST-π may, in part, play a role in scavenging of ROS generated by active metabolite of irinotecan hydrochloride - SN-38[64,70]. The metabolism of irinotecan hydrochloride and conversion to SN-38, is accompanied by carboxylesterases mediated hydrolysis. SN-38 targets Topoisomerase I, inhibiting DNA replication in cancer cells and leading to cell death. SN-38 is then glucuronised to SN-38 glucoronic acid and detoxified in the liver via conjugation by the

UGT1A1 family, which releases SN-38G into the intestines for elimination[71-74]. This metabolite contributes to the dose limiting toxicities such as: myelotoxicity, neutropenia, and diarrhoea[74].

It was also shown that the inhibition of MRP-1 promoter activity and impairment of MRP-1 function by indomethacin (Ann Arbor, MI, USA) resulted in the effect comparable to the effect of a potent non-competitive inhibitor of GST-π[75]. Indomethacin (a terminal half-life: 20 minutes) is rapidly metabolised by liver microsomes. CYP3A appears to be the primary enzyme involved in the oxidative metabolism of phenethyl amide side chain of indomethacin[76]. This drug increases chemotherapy sensitivity through inhibition of MRP1 function and stimulation of GSH efflux in MRP1-overexpressing cell lines[75]. This drug decreases mitochondrial GSH, which takes part into ROS inactivation. A reduction in cellular GSH level and elevated oxidative stress can be an important trigger for apoptosis induction[75,77]. It was demonstrated that oxidative stress resulted in the loss of mitochondrial membrane potential and release of several essential players of mitochondrial apoptosis pathway, such as cytochrome c and apoptosis-inducing factor into cytosol as well as activation of caspases and apoptotic protease-activating factor-1[75,78].

Actually, GST-π inhibitors are an important part in the design of anticancer drugs and treatment strategy that is aimed in particular to reverse cancer resistance[7,14,15,22,23,45,79]. GST is considered as an important factor influencing resistance of cancer cells to chemotherapeutic agents[80]. In order to inhibit the excessive activity of glutathione S-transferases, directed specific inhibitors thereof are designed or π isoform of GST are used to activate prodrugs targeted at cancer cells[14].

Taking into consideration the fact that most of anticancer drugs are not physiological substrates for GST, prodrugs may contain glutathione or glutathione-like structures. GSH analogues are obtained through modifications on γ-glutamyl, cysteine and glycine moieties, and/or central cysteine functionalisation, that are able to inhibit GSTs activity[7,81]. It has been found that during prodrugs activation, a conjugate with GSH is formed indirectly[14].

The first category of prodrugs contains the compounds of GSH or GSH-like structure, for which the β-elimination reaction, catalysed by GST, leads to cytotoxic metabolites release. This group of prodrugs includes nitrogen mustards that have the ability to alkylate DNA after further transformation, for example canfosfamide (TLK286,Telcyta) (a mean half-life: 18 min) and its analogues[82,83]. The detailed mechanism of GST-π activation of the prodrug has not been elucidated. It was suggested that a catalytic water molecule is instrumental for the prodrug activation by formation of a network of intermolecular interactions between the active-site Tyr7 hydroxyl, sulfone and COO(-) groups of canfosfamide[82]. The activation of this drug leads to alkylating metabolite production that forms covalent linkages with nucleophilic centres in tumour cell DNA. Canfosfamide is able to inhibit a DNA-dependent protein kinase, i.e. an enzyme involved in double strand break DNA repair. The upregulation of this enzyme leads to resistance to other anti-cancer drugs, including, but not limited to cisplatin, suggesting that canfosfamide can be used in combination with this drug[84]. It may induce a cellular stress response, cytotoxicity and decrease tumour proliferation. The phase I study reported that canfosfamide is well tolerated with mild and moderate adverse effect such as nausea, fatigue, vomiting or anaemia[85].

The other complex of prodrugs includes several groups of compounds that are non-structurally related to GSH, such as diaryl sulfonylureas (LY 186641: Windlesham, Surrey, U.K.[86], a half-life: about 30-40 hours), exocyclic enones (COMC-6: synthesised as reported by Hamilton et al[87]) and electrophilic diazenium diolates (JS-K: Santa Cruz, CA, USA[88], a half-life: about 20 hours)[14]. As a result of the reaction, it is possible to form GST-π competitive inhibitors[89], carbonylating agents[90] or cytotoxic agents such as nitric oxide that are subject to catalysis[91,92]. The chemotherapeutic effect is achieved by GSH consumption, induction of DNA double-strand breaks and activation of apoptosis pathways[92]. The antitumor and toxicological mechanisms of action of these drugs are not well understood, but unlike the other antineoplastic agents, sulofenur does not interfere with DNA, RNA, or protein synthesis, or with polynucleotide function. Most sulfonylureas are extensively metabolised in the liver, primarily by cytochrome P450 (CYP) 2C9 isoenzyme[93]. Sulofenur undergoes bioactivation *in vivo* to generate p-chlorophenyl isocyanate (CPIC), which can carbamoylate biological macromolecules directly or form a conjugate with GSH which serve as a latent form of CPIC. It was suggested that generation of this isocyanate *in vivo* and subsequent carbamoylation of biological macromolecules may play a role in toxicity and/or antitumor activity of sulofenur and related diarylsulfonylureas[94]. Ad-

verse effects of sulfonylureas traetment include hypoglycemia, cholestatic jaundice, skin rash, hemolytic anaemia, thrombocytopenia, agranulocytosis, flushing and hyponatremia[95].

According to the studies conducted on mouse fibroblasts, it was shown that the selective peptidomimetic inhibitor of GST-π, i.e. ezatiostat hydrochloride (Telintra®, TLK199, a half-life: 0.20 hours), after intracellular de-esterification to TLK1117, binds to and inhibits GST-π as a negative regulator of JNK kinases[4]. Disruption of the binding of GSTP1-1 to JNK leading to their activation and restoring Jun kinase and MAPK pathway, activities and promotes MAPK-mediated cellular proliferation and differentiation pathways[96]. It promotes proliferation and maturation of hematopoietic precursor cells, granulocytes, monocytes, erythrocytes and platelets, which allow this drug use in the therapy of hematologic disorders[96]. Treatment with the application of ezatiostat hydrochloride is associated with the occurrence of side effects such as chills, back and bone pain, flushing, nausea, fatigue, extremity pain, dyspnoea, and diarrhoea[97]. Another GST inhibitor, 6-(7-nitro-2,1,3-benzoxadiazol-4-ylthio) hexanol (NBDHEX: synthesised, as reported by Ricci et al[98]), eliminates the interaction between GST-π and JNK1α2. It was found that this process results in dissociation of the formed heterocomplex and further, leads to caspase-dependent apoptosis of various tumour cell lines[4]. Interestingly, it was shown that leukaemia MDR1-expressing cells demonstrated a higher degree of apoptosis and caspase-3 activity than their drug-sensitive counterparts[99]. The increased susceptibility of the multidrug resistance cells towards NBDHEX action may be related to a lower content of GST-π[99].

In the treatment of cancer, such as acute promyelocytic leukemia, the phenylarsine oxide (PAO: St. Louis, MO, USA), i.e. an organic derivative of arsenic is used. This compound can bind to GST-π even without GSH participation[22]. PAO interacts with reactive cysteine residue at position 47 of polypeptide chain in π isoform of GST. It changes the conformation of the region containing this cysteine and significantly widens the V-shaped dimer gap[22]. It was also shown that PAO may react with reactive cysteine residues at position 101 of the polypeptide chain on both enzyme subunits in the presence and absence of GSH. It was investigated that PAO and GSH adducts have the ability to bind themselves in the active centre of the enzyme in the form of di-GSH-phenyraxene complex. This form of PAO is considered as one of the most potent GST-π inhibitors[22]. It was also demonstrated that 8-methoxypsoralen (St. Louis, MO, USA, a half-life about 4 hours) can act as a GST-π inhibitor. Molecular docking simulations suggest that this compound can bind to the enzyme active site, but its position prevents GSH conjugation and impairs GST-π function[80].

It is believed that GST-π inhibitors are promising prototypes for new drugs that are useful in pharmacology in the case when cancer resistance to chemotherapy. The recent findings revealed an interesting possibility to use siRNA, as a factor silencing GST-π[100]. It was shown that GST-π silencing led to a significant impairment in the growth of cancer cells due to oxidative stress caused by excess ROS accumulation and facilitation of mitochondrial dysfunction[100]. These findings are promising for the development of strategies towards cancer therapy, based on the mechanism that allows genetic silencing of GST-π to promote oxidative stress through mitochondria dysfunction. It makes possible to avoid the problem of drug resistance, which will be solved at the transcriptional level[100].

Conclusions

GST-π is characterised by pleiotropic effects on the cells[4]. In physiological conditions, this enzyme is able to catalyse the S-glutathionylation of many proteins, including transcription factors. GST-π class has the ability to inactivate the p38 kinase signalling pathway (p38/MAPK/HSP27), thereby limiting cellular apoptosis. GST-π can weaken the effect of TNF receptor-associated factor 2 (TRAF2) on apoptosis signal-regulating kinase-1 by inactivation of MAP kinase pathways, such as c-Jun N-terminal and p38 kinases. Additionally, GST-π, as an enzyme involved in detoxifying processes, is engaged in lipids mediators metabolism, e.g. 15d-PGJ2 metabolism. This process limits the effect of 15d-PGJ2 activating PPARs, and then inhibits activation of cellular pathways leading to apoptosis.

An increased GST-π activity weakens the efficacy of drugs by promoting their efflux from the cells. Therefore, inhibition of GST-π activity and its overexpression (at the level of protein and transcript) in tumour lines have become a potential goal in therapy. The chemotherapeutic effect is achieved by GSH consumption, induction of DNA double-strand breaks and activation of apoptosis

pathways. Both non-competitive and direct inhibitors of GST-π are used in anticancer therapy. GST-π isoenzyme oxidation and its activity inhibition, can result in braking of TRAF2-GST-π complexes formation and unblocking cancer cells apoptosis. Additionally, a decrease in GST-π activity can enhance concentration of lipids mediators and their ability to activate PPARγ, as well as, following the aforementioned processes, inhibition of gene transcription, decreased DNA synthesis and orientation of cellular pathways to apoptosis. On the other hand, the use of inhibitors of proteins that are functionally-associated with GST-π, such as inhibitors of MRP pumps function, can also result in drug efflux suppression in cancer cells via MRP-1 function impairment. Therefore, GST-π is recognised as an important target in anticancer therapy, what is used in designing new anticancer drugs. Anticancer drugs are often substrates for GST-π or have the affinity with its structures which results in weakening its activity and achieving therapeutic goal.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Authors' Contribution

The idea for this article belongs to Halina Milnerowicz. The literature search, data analysis and draft of this article were performed by Milena Ściskalska. The article was critically revised by Halina Milnerowicz.

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