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THE ROLE OF GLUTATHIONE TRANSFERASES IN RENAL CELL CARCINOMA

ULOGA GLUTATION TRANSFERAZA U KARCINOMU BUBREŽNOG PARENHIMA

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ABSTRACT

Mounting evidence suggest that members of the subfamily of cytosolic glutathione S-transferases (GSTs) possess roles far beyond the classical glutathione-dependent enzymatic conjugation of electrophilic metabolites and xenobiotics. Namely, monomeric forms of certain GSTs are capable of forming protein: protein interactions with protein kinases and regulate cell apoptotic pathways. Due to this dual functionality of cytosolic GSTs, they might be implicated in both the development and the progression of renal cell carcinoma (RCC).

Prominent genetic heterogeneity, resulting from the gene deletions, as well as from SNPs in the coding and non-coding regions of GST genes, might affect GST isoenzyme profiles in renal parenchyma and therefore serve as a valuable indicator for predicting the risk of cancer development. Namely, GSTs are involved in the biotransformation of several compounds recognized as risk factors for RCC. The most potent carcinogen of polycyclic aromatic hydrocarbon diol epoxides, present in cigarette smoke, is of benzo(a)pyrene (BPDE), detoxified by GSTs. So far, the relationship between GST genotype and BPDE-DNA adduct formation, in determining the risk for RCC, has not been evaluated in patients with RCC.

Although the association between certain individual and combined GST genotypes and RCC risk has been debated in a the literature, the data on the prognostic value of GST polymorphism in patients with RCC are scarce, probably due to the fact that the molecular mechanism supporting the role of GSTs in RCC progression has not been clarified as yet.

Keywords:
Glutatione S-transferase,
polymorphism,
renal cell carcinoma

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SAŽETAK

Veliki broj dokaza govori u prilog tome da pojedini pripadnici citosolnih glutation S-transferaza (GST) poseduju i uloge nezavisne od njihove klasične uloge u konjugaciji elektrofilnih metabolita i ksenobiotika sa glutationom. Naime, monomerni oblici pojedinih GST sposobni su da formiraju protein: proteinske interakcije sa izvesnim protein-kinazama i time regulišu puteve proliferacije i preživljavanja u ćeliji. Smatra se da zbog ove svoje dvostruke uloge citosolne GST mogu da utiču kako na nastanak, tako i na progresiju karcinoma bubrežnog parenhima (KBP).

Značajna genetska heterogenost, nastala ili kao rezultat delecije gena ili usled prisustva polimorfizma jednog nukleotida, kako u kodirajućim, tako i nekodirajućim sekvencama *GST* gena, može da utiče na GST izoenzimski profil u bubrežnom parenhimu i posluži kao dragoceni pokazatelj za procenu rizika za nastanak karcinoma. Glutation transferaze su uključene u reakcije biotransformacije nekoliko jedinjenja priznatih kao faktori rizika za KBP. Benzo(a)piren diol-epoksid (BPDE) pripada grupi diol-epoksida iz grupe policikličnih aromatičnih ugljovodonika i, kao jedan od najopasnijih kancerogena prisutnih u duvanskom dimu, supstrat je za pojedine GST. Odnos između GST genotipa i nivoa BPDE-DNK adukta do sada nije analiziran u svetlu procene rizika za nastanak KBP.

Iako je veza između određenih pojedinačnih i kombinovanih *GST* genotipova i rizika za nastanak KBP bila predmet analiza velikog broja radova, nema puno podataka koji govore u prilog prognostičkom značaju *GST* polimorfizma kod pacijenata sa KBP, verovatno zbog činjenice da molekularni mehanizam, koji je u osnovi uloge GST u progresiji KBP, nije još uvek razjašnjen.

Ključne reči:
glutation S-transferaza, polimorfizam, karcinom bubrežnog parenhima

RENAL CELL CARCINOMA (RCC)

Renal cell carcinoma (RCC) is the predominant form of kidney malignancy, comprising a group of heterogeneous renal tumors (1,2), with the clear cell RCC (ccRCC) being the most frequent subtype of sporadic RCC in adults (70-85%) (3,4).

In 2013, kidneys were recognized as the seventh most common site for tumor development (5). Renal cell carcinoma is the predominant form of kidney malignancy, whereas urothelial carcinoma, arising in the renal pelvis, accounts for less than 10% of histologically confirmed kidney carcinomas. (2). In 2012, the global incidence rate reported for RCC was 6.0/100.000 for men and 3.0/100.000 for women (5). Similarly, in 2013 the incidence in Serbia was reported as 6.1 (men) and 3.0 (women) per 100.000 people (6).

Most RCC are asymptomatic. It seems that the use of high-resolution cross-sectional imaging modalities over the last few decades has led to the increase in incidental detection of renal masses, often characterized as small, and low-graded (7). Nowadays, between 48-66% of such RCCs are detected incidentally (8). Still, many renal masses remain asymptomatic until the late stages of the disease. Despite advances in diagnostic methods, about 20-30% of patients are diagnosed with metastatic disease and 20% of patients undergoing nephrectomy will eventually develop metastatic RCC during the follow up period (9,10).

Cigarette smoking, obesity and hypertension are the most well established risk factors for sporadic RCC (2,10-13). Cigarette smoke is a rich source of free radicals, which are believed to be responsible for initiation of many tumors by inducing DNA damage that accumulates in cells. In addition to free radicals, more than 60 carcinogens have been found in cigarette smoke. Among these, sufficient evidence of carcinogenicity was found for polycyclic aromatic hydrocarbons (PAHs), such as benzo(a)pyrene and aromatic amines, such as 4-amino biphenyl (14). Particular interest has been given to the most abundant, benzo(a)pyrene (B(a)P) and its carcinogenic metabolites, stereoisomers of 7,8-dihydroxy-9,10-oxy-7,8,9,10-tetrahydrobenzo(a)pyrene (BPDE) (15). The (+)-antiisomer [(+)-anti-BPDE] seems to be the most potent carcinogen of all PAH diol epoxides (16). Namely, BPDE is known as 'the bullet of the smoking gun', leaving its fingerprints in the blood of smokers, in the form of adducts with either serum albumins or DNA (15,17).

In recent years, the genetic origin of RCC became a focus of research, since not all individuals exposed to recognized RCC risk factors develop renal cell carcinoma. In general, an effort has been made towards identifying the common genetic variations, known as "quantitative trait loci", that could contribute a small, but significant risk not only for the development, but for the progression of complex disorder such as cancer (18).

GLUTATHIONE S- TRANSFERASES (GSTs)

A growing number of genes encoding enzymes involved in biotransformation and cellular defense has been identified, leading to increased knowledge of allelic variants of genes that may result in a differential susceptibility to environmental and oxidative stress (19,20). Glutathione transferases (EC 2.5.1.18), also referred to as glutathione

S-transferases or GSTs, are members of a multi-gene family. There are three major families of GST proteins, with cytosolic GSTs constituting the largest family (21). Seven classes of cytosolic GSTs have been identified in mammals (22), comprising a set of cellular proteins (GSTome) with various catalytic and non-catalytic functions (23,24). Namely, primary metabolic role of GST is to detoxify reactive electrophiles, such as potent xenobiotic, carcinogens and even therapeutic drugs (20), by catalyzing reaction of conjugation with glutathione. Glutathione conjugates are, thereafter, exported from the cell and subjected to metabolism of mercapturic acid, followed by the excretion in the urine (25) or bile (26). Thereby, GSTs reduce the likelihood of deleterious interactions of reactive compounds with important cellular macromolecules, such as proteins and nucleic acids (27).

However, not all reactions catalyzed by GST enzymes result in detoxification. Namely, in certain instances some GSTs are associated with bio-activation of electrophilic compounds (28,29) where the glutathione conjugate is more reactive than the parent compound. A growing number of evidence supports the aforementioned phenomenon, where mutagens, carcinogens and even some prodrugs are metabolically activated by conjugation with GSH (28,30). There are evidence that this is particularly true for the kidney (31,32).

Being a multifunctional group of enzymes, GSTs are involved in, intracellular binding and transport of hydrophobic compounds (33), and catalysis of key steps in the synthesis of leukotrienes, prostaglandins (34), steroid hormones (35), as well as the degradation of tyrosine (21). Moreover, some GST isoenzymes exhibit selenium independent glutathione peroxidase activity and along with other antioxidant enzymes provide a certain shield against a range of harmful electrophiles, produced during redox imbalance (36). Phospholipid, fatty acid and cholesterol hydroperoxides seem to be substrates for several GSTs, especially for the members of class *alpha* enzymes (37).

In addition to their role in the biotransformation reactions, there are evidence which clearly indicate the involvement of GST in the cellular survival, proliferation and apoptosis as well, by the means of protein: protein interactions with the signaling molecules, such as mitogen activating protein kinases (MAPK) (19,22,38,39). The first example of GST-mediated kinase regulation was the discovery of the GSTP1:JNK1 complexes (40). Namely, it seems that under physiological conditions, a portion of GSTP1 is bound to c-Jun NH2-terminal kinase (JNK1), regulating the level of JNK1 activity. However, in case of increased reactive oxygen species content, the GSTP1:-JNK1 complex dissociates which in turn leads to the association of GSTP1 into oligomers. Now activated, JNK1 induces a chain of events, starting from the phosphorylation of its substrate, the transcription factor c-Jun, and resulting in apoptosis (19,40).

Another example of protein: protein interaction, similar to those of GSTP1, is a complex between mitogen activated kinase (MAPK) ASK1 and GSTM1-1, found to be important for the maintenance of the normal level of p38 phosphorylation (41). Namely, ASK1 is MAPK kinase that activates JNK1 and p38 pathways, leading to cytokine and stressed-induced apoptosis (42). Environmental stress causes the disruption of the complex of GSTM1:ASK1, which accumulates GSTM1 into oligomers, while ASK1 is being activated (43). This dissociation results in a subsequent activation of JNK1 and p38-dependent signal pathways, ultimately leading to stress-induced apoptosis. In particular, *Cho et al., 2001* (41) showed both *in vitro* and *in vivo,* that mouse glutathione S-transferase Mu 1-1 (mGSTM1-1) physically interacts with ASK1 and, in doing so, functions as a negative regulator of ASK1 inside cells, repressing ASK1-mediated signals.

Genetic variations in human GSTs

Deletions and single-nucleotide polymorphisms (SNP) occur in genes encoding for members of the glutathione S-transferase superfamily (GSTs; EC 2.5.1.18), resulting in complete lack or alteration in enzyme activity (44).

Both *GSTM1* and *GSTT1* genes exhibit homozygous deletion polymorphisms, commonly referred to as *the null genotype*. The general lack of enzymes in such individuals has been recognized as potentially important modifier of individual risk for environmentally induced cancers (44). In case of *GSTM1-null* genotype, the underlying mechanism conferring an increased risk of cancer would be that such individuals are more susceptible to chemical-induced carcinogenesis, due to the diminished activity of xenobiotic-metabolizing defense system (45). On the other hand, it seems that when it comes to gene-environment interactions, GSTT1 deficiency may be either deleterious or beneficial depending upon substrate exposure. Namely, members of the GST *theta* class are involved in bio-activation of certain compounds, producing even more toxic reactive intermediates, as a result of GSH conjugation (46).

Contrary to other GSTs, several SNPs have been identified in 5' non-coding promoter region of *GSTA1* gene, among them *GSTA1**C69T (rs3957356), reducing the levels of GSTA1 enzyme in carriers of the variant *GSTA1**B in liver (Coles and Kadlubar, 2005). This *GSTA1* polymorphism is represented by three, apparently linked, SNPs: T-567G, C-69T and G-52A located within in the proximal promoter (47). It has been suggested that this genetic variation of GSTA1 can change an individual's susceptibility to carcinogens and toxins, as well as affect the efficacy of some drugs (48).

GSTP1 SNP (rs1659) is one of the most extensively studied *GST* polymorphisms. This SNP encodes *the Ile-105Val* substitution, which influences *Ile105* and *Val105* variants' catalytic efficacy (49,50) and has been investigated not only in terms of cancer susceptibility, but also in relation to drug resistance (22,51,52). It has been shown that *Ile105Val* substitution contributes to the architecture

of the hydrophobic substrate binding GST site and different substrate specificity (53). For instance, GSTP1 variants exhibit significantly different rates of conjugating activity towards (+)-anti-BPDE, with higher turnover for isoform *GSTP1**Val105 than for isoform *GSTP1**Ile105, due to the more favorable substrate-binding setting (50).

GENETIC POLYMORPHISM OF GLUTATHIONE TRANSFERASES IN PATIENTS WITH RENAL CELL CARCINOMA

A growing body of evidence suggests that cytosolic GSTs might be implicated not solely in the development, but also in the progression of RCC (54-57). The GSTs are involved in the biotransformation of several compounds recognized as risk factors for RCC (44). The main site for the initial glutathione conjugation of toxic compounds is generally assumed to be the liver, followed by a mandatory transfer of conjugates to the kidney (58). However, the initial bio-activation step of some nephrocarcinogens can take place in the kidney itself (59). The potential genotoxicity of carcinogens depends on the biotransformation capacity of renal tissue. Prominent genetic heterogeneity, resulting from the gene deletions, as well as from SNPs in the coding and non-coding regions of *GST* genes, might affect GST isoenzyme profile in renal parenchyma and, therefore, serve as a valuable indicator for predicting the risk of cancer development (45).

The deletion of *GSTM1* gene is one of the most investigated *GST* polymorphisms, since it has been suggested that a common variation within the *GSTM1* gene can modify susceptibility to various cancers, including renal cell carcinoma (44,45). Indeed, it has been demonstrated that the carriers of *GSTM1-null* genotype are in significantly higher risk of developing ccRCC compared to the carriers of *GSTM1-active* genotype (60).

GSTT1 deficiency is also a result of the gene deletion. After it has been discovered that GSTT1 could catalyze activation of certain compounds to even more reactive intermediates (28,29,31), the *GSTT1* deletion polymorphism was the subject of many studies, some of which tried to establish whether the presence of the *GSTT1-active* genotype was associated with RCC development, independently or in combination with exposure to certain environmental or occupational hazards (32,61,62). However, the results available in the have shown that *GSTT-1*genotype does not, at least independently, affect the susceptibility to RCC (60,63).

The expression of *GSTA1* is exclusively observed in clear cell RCC (58), while in RCC of the chromophobic cell type this protein is completely absent (64). However, the data on the potential role of *GSTA1* SNP in both onset and prognosis of RCC are limited, showing the lack of association in terms of increased risk for RCC development (60,65).

Although the certain meta-analyses on *GST* polymorphisms in RCC did not report any individual association between *GSTP1* genotype and RCC (63,66), recent results indicated that *GSTP1-variant (ValVal)* genotype was associated with a significant individual risk for ccRCC development, that was even more pronounced in combination with other *GST* genotypes (60). Namely, if genetic susceptibility to RCC development is, at least partially, affected by polymorphisms in genes involved in xenobiotic metabolism, it is possible that the combinations of certain genotypes may be more discriminating as risk factor for RCC development than a single one. Interestingly, when association of combined *GST* genotypes was analyzed in terms of RCC risk, *GSTM1-null/GSTT1-active/GSTA1 low-activity/GSTP1-variant* genotype combination was recognized as "the *RCC risk carrying genotype*"(60).

Glutathione S-transferase (GST), xenobiotic-metabolizing enzymes, play an important role in protection from carcinogens. Presumably, *GST* genotyping could identify individuals in whom detoxification is diminished, due to complete lack or alteration in enzyme activity. Consequently, they are more likely to accumulate carcinogen-DNA-adducts and/or mutations, increasing their susceptibility to cancer development. Namely, DNA adducts associated with tobacco smoking have been suggested as a marker of biologically effective dose of tobacco carcinogens that might improve individual cancer risk prediction (67). Both free radicals and reactive polycyclic aromatic hydrocarbons metabolites, such as BPDE, are detoxified by GSTs (68,69). Indeed, it has been shown that the clear cell RCC smokers with *GSTM1-null* genotype had significantly higher concentration of BPDE-DNA adducts in comparison with *GSTM1-active* RCC smokers (60).

FUTURE PERSPECTIVES

Some studies suggest that cytosolic GST may be implicated not solely in the development, but also in the progression of RCC (55,70). Although the associations between the certain *GST* genotypes and RCC risk has been debated in the literature (63,66,70–73), the data on the prognostic value of *GST* polymorphism in patients with RCC are scarce (55), probably due to the fact that the molecular mechanism supporting the role of GSTs in RCC progression has not been clarified as yet. A possible underlying mechanism might be the regulation of one major signaling pathway, constituting mitogen-activated protein kinases (MAPK) by GSTs. According to the results obtained *in vivo* and *in vitro* setting, mouse GSTM1-1 physically interacts with ASK1, functioning as ASK1 negative regulator (41). It seems that the ASK1-JNK/p38 pathway is recognized as quite important in the occurrence of the apoptosis in RCC cells (74). Thus, the patients with *GSTM1-null* genotype and consequently deficient GSTM1, might have decreased tumor proliferation due to increased apoptotic activity, leading to slower RCC progression and better survival. On the other hand, RCC pa-

tients with *GSTM1-active* genotype may have lower ASK1 activity, resulting in decreased apoptotic activity in the tumor and poorer survival.

Moreover, monomeric GSTP1 subunits inhibit JNK1 by either blocking phosphorylation of JNK or by promoting dephosphorylation of phosphorylated JNK (75). In this manner, JNK is prevented from activating downstream targets in the apoptotic pathway, which might contribute to tumor progression or even drug resistance. Namely, with regard to this role, high tumor GSTP1 expression has been associated with drug resistance, failure of therapy and poor patient survival. Interestingly, GSTP1 overexpression has been found in drug resistant cells, even in instances where there is no evidence that the selecting drug is a substrate for GSTP1 (52).

So far, there are no data which would indicate the significance of GSTM1:ASK1 and GSTP1:JNK1 protein: protein interactions in human RCC in terms of tumor progression. What is more, it is still unclear whether the polymorphic expression of GSTM1 may influence the activity of apoptotic signal pathways in RCC progression.

CONCLUSION

Due to the potential functional significance of common polymorphisms in genes encoding cytosolic glutathione transferase A1, M1, T1 and P1, in both onset and prognosis of RCC, it might be speculated that the presence of specific *GST* gene variants in RCC patients is not only associated with the risk of RCC development, but might also affect the tumor progression and postoperative prognosis.

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