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THE RELEVANCE OF OMEGA CLASS GLUTATHIONE TRANSFERASE POLYMORPHISMS AND EXPRESSION IN DEVELOPMENT AND PROGRESSION OF CLEAR CELL RENAL CELL CARCINOMA

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POVEZANOST POLIMORFIZAMA I EKSPRESIJE GLUTATION TRANSFERAZA KLASE OMEGA SA NASTANKOM I PROGRESIJOM SVETLOĆELIJSKOG KARCINOMA BUBREŽNOG PARENHIMA

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ABSTRACT

THE RELEVANCE OF OMEGA CLASS GLUTATHIONE TRANSFERASE POLYMORPHISMS AND EXPRESSION IN DEVELOPMENT AND PROGRESSION OF CLEAR CELL RENAL CELL CARCINOMA

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Background: Novel omega class glutathione transferases, GSTO1-1 and GSTO2-2, possesses an intriguing variety of both catalytic and non-catalytic roles involved in regulation of inflammation, apoptosis and redox homeostasis. Two coding single nucleotide polymorphisms (SNPs), *GSTO1**C419A (rs4925) and *GSTO2**A424G (rs156697) supposedly affect their functions, whereas functional significance of other *GSTO2* polymorphism, found at the 5′ untranslated (5′UTR) gene region (*GSTO2**A183G, rs2297235), has not been clearly elucidated thus far. This study represents the first comprehensive research on the relevance of polymorphisms and expression profiles of GSTO class in the most aggressive renal cell carcinoma subtype (clear cell RCC, ccRCC). The potential effect of these polymorphisms was studied in regard to both risk and postoperative ccRCC prognosis. Furthermore, GSTO1-1 and GSTO2-2 expression, as well as phosphorylation status of downstream effectors in PI3K/Akt/mTOR and Raf/MEK/ERK signaling pathways in non-tumor and tumor ccRCC tissue were assessed. Possible association of GSTO1-1 with signaling molecules suggested to be regulated by glutathionylation was also studied.

Methods: Genotyping was achieved in 239 ccRCC patients and 350 matched controls. In ccRCC tumor and corresponding non-tumor tissue were assessed expression of GSTO1, GSTO2, and signaling molecules by Western blot. Biomarker of oxidative DNA damage, 8-hydroxy-2′-deoxyguanosine (8-OHdG) and cytosolic pro-IL-1β and IL-1β were determined by ELISA. Co- immunoprecipitation with GSTO1 was performed.

Results: Carriers of all three GSTO variant genotypes combined showed almost 3-fold risk of ccRCC development. Furthermore, this association was confirmed by the haplotype analysis. The H2 haplotype, comprising variant *GSTO1**A (rs4925), *GSTO2**G (rs156697) and *GSTO2**G (rs2297235) alleles, carried the highest ccRCC

risk, suggesting a possible role of those variants in cancer susceptibility. Regarding the gene-environment interactions, smokers with variant *GSTO2* (rs156697) genotype were at higher ccRCC risk in comparison with non-smokers carriers of at least one referent allele. Association concerning oxidative DNA damage was found for *GSTO2* rs2297235 polymorphism and 8-OHdG. After 7-years follow-up, it has been shown that *GSTO1**CC genotype predicts shorter survival of male ccRCC patients. Moreover, in the multivariate Cox regression analysis male carriers of *GSTO1**CC genotype had significantly increased hazard ratio compared to the carriers of *GSTO1**A allele. In addition to findings on both significant GSTO1-1 and GSTO2-2 upregulation in ccRCC, the change in expression levels of these two isoenzymes between early-stage and late-stage ccRCC was found. Expression of phosphorylated downstream effectors of Akt/MAPK signaling pathways (RSK1p90 (pS380), Akt (pS473) and ERK1/2 (pY204/187)) was also enhanced in ccRCC tumor in comparison with corresponding non-tumor specimens. What is more, GSTO1-1 immunoprecipitated with majority of investigated phosphorylated downstream signaling molecules, except ERK1/2.

Conclusions: In conclusion, the concomitance of GSTO polymorphisms may influence ccRCC risk, while prognostic role has only *GSTO1* polymorphism. Up-regulated GSTO1-1 and GSTO2-2 enzymes in ccRCC tumor tissue might contribute to aberrant redox homeostasis and tumor progression. The possible molecular mechanism might be partially explained by GSTO1-1 deglutathionylase activity.

Key words: clear cell renal cell carcinoma; GSTO1; GSTO2; polymorphism; haplotype; risk; survival; IL-1β; Akt; expression

Field: Medicine

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REZIME

POVEZANOST POLIMORFIZAMA I EKSPRESIJE GLUTATION TRANSFERAZA KLASE OMEGA SA NASTANKOM I PROGRESIJOM SVETLOĆELIJSKOG KARCINOMA BUBREŽNOG PARENHIMA

Tanja M. Radić

Uvod: Predstavnici nove omega klase glutation transferaza, GSTO1-1 and GSTO2-2, nosioci su kako katalitičkih tako i nekatalitičkih uloga značajnih u regulaciji procesa poput inflamacije, apoptoze i redoks homeostaze. Kodirajući polimorfizmi jednog nukleotida *GSTO1**C419A (rs4925) i *GSTO2**A424G (rs156697) po svoj prilici utiču na funkciju proteina dok funkcionalna značajnost još jednog *GSTO2* polimorfizma, identifikovanog na 5′ netranslatiranom regionu gena (*GSTO2**A183G, rs2297235), još uvek nije jasno rasvetljenja. Ova studija predstavlja prvo sveobuhvatno istraživanje povezanosti polimorfizama i ekspresije GST klase omega u jednom od najagresivnijih tipova karcinoma bubrežnog parenhima (svetloćelijski tip carcinoma bubrežnog parenhima, sKBP). Potencijalni uticaj ovih polimorfizama je izučavan kako u odnosu na rizik za nastanak sKBP tako i u odnosu na postoperativnu prognozu ovih pacijenata. Pored toga, ispitivana je i ekspresija GSTO1-1 i GSTO2-2 kao i fosforilacioni status nizvodnih efektorskih molekula u okviru PI3K/Akt/mTOR i Raf/MEK/ERK signalnih puteva u tumorskom i u netumorskom tkivu pacijenata sa sKBP. Moguća interakcija GSTO1-1 sa signalnim molekulima, za koju se pretpostavlja da je regulisana glutationilacijom, takodje je bila predmet izučavanja ove studije.

Materijal i metode: Polimorfizmi *GSTO1* i *GSTO2* gena određivani su kod 239 pacijenata sa sKBP i 350 pripadnika kontrolne grupe, uparenih po godinama i polu. Ekspresija GSTO1, GSTO2 i signalnih molekula je analizirana metodom imunoblota, kako u tumorskom tako i u netumorskom tkivu pacijenata sa sKBP. Nivoi pokazatelja oksidativnog oštećenja DNK, 8-hidroksi-2-deoksiguanozina (8-OHdG) i citosolnog prol-IL-1β i IL-1β su određivani ELISA metodom. Interakcije GSTO1 sa pojedinim signalnim molekulima su ispitivane metodom imunoprecipitacije.

Rezultati: Nosioci kombinovanih varijantnih genotipova su bili u 3 puta većem riziku od nastanka sKBP. Ova asocijacija je dodatno potvrdjena na nivou analize hapoltipa.

Nosioci haplotipa H2 koji podrazumeva varijantne *GSTO1**A (rs4925), *GSTO2**G (rs156697), i *GSTO2**G (rs2297235) alele, imali su najveći rizik za nastanak sKBP, ukazujući na potencijalnu ulogu ovih varijantnih alela na podložnost za nastanak sKBP. Analiza interakcije genotipova i faktora spoljašnje sredine ukazala je da su pušači nosioci *GSTO2**G (rs156697) varijantnog genotipa u većem riziku od nastanka sKBP u poređenju sa nepušačima nosiocima bar jednog referentnog alela. Pokazana je udruženost *GSTO2* polimorfizma u 5′ netranslatiranom regionu gena i nivoa 8-OHdG (p=0,042). Nakon sedmogodišnjeg praćenja preživljavanja pacijenata sa sKBP, pokazano je da *GSTO1**CC genotip može biti prediktor kraćeg preživljavanja kod muškaraca sa sKBP. Pored toga, multivarijantna *Cox* regresiona analiza je pokazala da su muškarci, nosioci *GSTO1**CC genotipa imali statistički značajno veći rizik od smrtnog ishoda u poređenju sa nosiocima *GSTO1**A alela. Pored povećane ekspresije GSTO1-1 i GSTO2-2 u tumorskom tkivu, uočena je različita ekspresija ova dva izoenzima kod pacijenata sa ranim stadijumom bolesti u odnosu na kasni stadijum bolesti. Ekspresija fosforilisanih nizvodnih efektorskih signalnih molekula Akt/MAPK signalnog puta (RSK1p90 (pS380), Akt (pS473) i ERK1/2 (pY204/187)) je takođe bila povišena u tumorskom u poređenju sa netumorskim tkivom. Šta više, nadjena je interakcija GSTO1-1 sa većinom ispitivanih nizvodnih efektorskih signalnih molekula, osim sa ERK1/2 molekulom.

Zaključci: Određeni polimorfizmi pripadnika omega klase GST mogu imati značajan efekat na rizik za nastanak sKBP, dok je prognostičku ulogu ima samo *GSTO1* polimorfizam. Povećana ekspresija GSTO1-1 i GSTO2-2 enzima u sKBP može doprineti narušenoj reodoks homeostazi i progresiji tumora. Deglutationilišuća aktivnost GSTO1-1 bi mogla biti molekularni mehanizam koji doprinosi ulozi ovog enzima u progresiji sKBP.

Ključne reči: svetloćelijski karcinom bubrežnog parenhima; GSTO1; GSTO2; polimorfizam; haplotip; rizik; preživljavanje; IL-1β; Akt; ekspresija

Naučna oblast: Medicina

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Contents

1 INTRODUCTION

1.1 Renal cell carcinoma

Renal cell carcinoma (RCC) comprises a heterogeneous group of cancers, derived from renal tubular epithelial cells (Eble et al., 2006) and accounts for 2%–3% of all new adult malignancies (Bamias et al., 2017). Lately, the incidence of RCC has increased with approximately 209 000 new cases per year over the world (Escudier et al., 2014), still being very high in the Czech Republic, Baltic and eastern European countries (Znaor et al., 2015). The median age at diagnosis for both sexes is 64 years (Rini et al., 2009), however, men are more prone to disease than women (a 2:1 ratio) (Hsieh et al., 2017b). Contrary to higher incidence rates, mortality rates have been decreasing in past decades, particularly in developed countries (Hsieh et al., 2017b).

Due to improvement of histopathological and molecular characterization of RCC, major revisions of RCC classification have been introduced in recent years (Hsieh et al., 2017b). Among more than 10 subtypes of RCC, cancers with the highest incidence are clear cell RCC (ccRCC, ~75%), papillary RCC (pRCC, ~15%) and chromophobe RCC (chRCC, ~5%) (Cancer Genome Atlas Research Network, 2013; Chen et al., 2016; Davis et al., 2014; The Cancer Genome Atlas Research Network, 2016). With clear cell subtype being predominant in metastatic disease (83–88%), all other types have been grouped as non-clear-cell RCC (nccRCC) (Hsieh et al., 2017b). Moreover, ccRCC is the most common and the most aggressive subtype is ccRCC accounting for most RCC deaths (Rini et al., 2009). Comprehensive investigations of genetic alterations (Cancer Genome Atlas Research Network, 2013; Hsieh et al., 2017b; Sato et al., 2013) have shown significant intra-tumor and inter-tumor genetic diversity in ccRCC, whose development and progression are characterized by genetic, epigenetic and proteomic changes, all contributing to heterogeneity of clinical outcomes (Hsieh et al., 2017a).

1.1.1 Risk factors

1.1.1.1 Modifiable risk factors

The major established risk factors for RCC include obesity, cigarette smoking, and hypertension (Lipworth et al., 2009). Moreover, incidence of RCC seems to be higher among patients with end-stage renal disease, kidney transplantation, acquired

kidney cystic disease, tuberous sclerosis, and, possibly, diabetes mellitus (Hsieh et al., 2017b; Rini et al., 2009).

Approximately 30% of RCCs in Europe are estimated to be attributable to being overweight and obese (Lipworth et al., 2009). Multiple studies have shown that increased body mass index (BMI) and abdominal obesity (measured as waist-to-hip ratio) were associated with RCC, particularly among women (Lipworth et al., 2009). It has been suggested that obesity-promoted changes in steroid hormones, elevated cholesterol and adipose tissue-derived hormones levels could contribute to RCC development (Lipworth et al., 2009). What is more, obesity seems to be associated with increased lipid peroxidation resulting in formation of DNA adducts, which could also contribute to RCC development (Gago-Dominguez et al., 2002).

Cigarette smoking is an established risk factor for RCC, however, with moderate effect on disease development which, on the other hand, seems to be dose-dependent. The proportion of RCC attributable to cigarette smoking is 20% to 30% among men and 10% to 20% among women (Lipworth et al., 2009).

Immense number of studies have reported association of hypertension with increased RCC risk ranging between 1.2 and 2 or greater (Lipworth et al., 2009). Increased risk was even reported in the studies that excluded early years of follow-up since early stage tumors may themselves cause higher blood pressure (Lipworth et al., 2009; Weikert et al., 2008; Yuan et al., 1998).

1.1.1.2 Genetic risk factors

Genetic factors also contribute to RCC risk. Studies of familial RCC found mutations in at least 11 genes (*BAP1*, *FLCN*, *FH*, *MET*, *PTEN*, *SDHB*, *SDHC*, *SDHD*, *TSC1*, *TSC2* and *VHL*), some of which have also been identified in the development of sporadic RCC (Haas and Nathanson, 2014; Hsieh et al., 2017b). Mutated *VHL* gene, otherwise encoding for pVHL, underlies von Hippel–Lindau disease, which is associated with a high risk of ccRCC development (Hsieh et al., 2017b). Additionally, genome-wide association studies (GWAS) of RCC have identified several susceptibility loci, located on chromosome regions 2p21, 2q22.3, 8q24.21, 11q13.3, 12p11.23 and 12q24.31 (Hsieh et al., 2017b). Particularly, the 2p21 locus comprises *EPAS1*, which encodes the hypoxia-inducible factor 2α (HIF2α) subunit (Purdue et al., 2011), while the

biological effects of mutations in 11q13.3 locus might be associated with changes in the regulation of *CCND1* encoding cyclin D1, which is involved in cell cycle regulation (Hsieh et al., 2017b; Schödel et al., 2012).

1.1.2 Diagnosis

Due to asymptomatic presentation of most RCCs, historically, patients were diagnosed after appearance of a palpable abdominal mass, flank pain, gross haematuria, metastatic symptoms (bone pain or lung nodules) and paraneoplastic syndrome (hypercalcaemia, fever and erythrocytosis) (Rini et al., 2009). Widespread use of non-invasive radiological techniques, such as ultrasonography, abdominal computed tomography (CT) and magnetic resonance imaging (MRI) significantly increased detection of small renal masses with low grade (Gill et al., 2010). In contrast to prior assessment of RCC diagnosis in the late stages of disease, nowadays, more than 50% of RCCs are detected incidentally (Bamias et al., 2017). Diagnosis is usually suggested by ultrasonography and further investigated by CT scan for assessment of local invasiveness, lymph node involvement or other metastases. MRI may be useful when it is not possible to use i.v. contrast and also may provide additional information in examining local advancement. Abdominal and chest CT or MRI is obligatory to achieve accurate staging of RCC. Positron emission tomography is not a standard investigation in the diagnosis and staging of RCC (Escudier et al., 2014).

The stage of RCC considers the tumor size, the degree of invasion outside of the kidney, the involvement of lymph nodes and presence of metastases. Prognostic evaluation involves further laboratory testing consisting of determination of haemoglobin levels, leukocyte and platelet counts, serum-corrected calcium levels and lactate dehydrogenase activity (Hsieh et al., 2017b).

1.1.3 Therapy

Localized RCC can be treated with radical or partial nephrectomy which offers lower post-operative renal function damage. Alternative strategies for patients that are unsuitable for surgery due to health complications are ablation or active surveillance (monitoring of tumor growth). Even after nephrectomy, approximately 30% of patients with localized ccRCC will develop metastases (Hsieh et al., 2017b). Systemic therapies

necessary for the treatment of metastatic disease have been developed in recent years as a result of comprehension of molecular mechanisms underlying metastatic ccRCC. Targeted therapies against vascular endothelial growth factor (VEGF) (sorafenib, sunitinib, pazopanib, axitinib, lenvatinib and cabozantinib) and mechanistic target of rapamycin (mTOR) pathways (everolimus and temsirolimus) have been developed (Hsieh et al., 2017b).

1.1.4 Molecular hallmarks of ccRCC

1.1.4.1 Genetics of ccRCC

Initial steps towards comprehension of ccRCC genetics comprised von Hippel Lindau (VHL) disease studies. VHL disease is an autosomal-dominant syndrome predisposing disease-bearing individuals to a diversity of benign and malignant neoplasms (Haddad and Margulis, 2015). Such individuals carry a germline mutation of the *VHL* tumor suppressor gene where the somatic inactivation or loss of the second wild-type allele determines tumor development. Indeed, *VHL* gene inactivation either through somatic mutation (such as single base modifications, insertions, deletions, as well as 3p25 loss) and/or promoter hypermethylation is found in approximately 90% cases of sporadic non-hereditary ccRCC. The *VHL* gene encodes for VHL protein (pVHL), the substrate recognition component of an E3-ubiquitin ligase complex, responsible for ubiquitylation of hypoxia-inducible factor α proteins (HIF1α and HIF2α) leading to degradation mediated by proteasome under normoxic conditions (Hsieh et al., 2017b; Masson and Ratcliffe, 2014; Semenza, 2013). Therefore, pVHL loss of function induces abnormal accumulation of HIFα proteins in normoxia, ensuing downstream overexpression of HIF-targeted genes. Among them, particularly important are those genes involved in regulation of complex biochemical and cellular events such as angiogenesis, proliferation, invasion, metabolism of glucose and survival (Mehdi and Riazalhosseini, 2017). Analogous to hypoxia-induced cascade, these events might explain the pseudohypoxic model of renal tumorigenesis (Bratslavsky et al., 2007).

Although heterogeneous by etiology, pVHL inactivation is found in 90% of all ccRCC patients, however, insufficient to induce ccRCC (Sanchez and Simon, 2018). Long latent period of more than 3 decades in humans carrying *VHL* inherited mutations, as well as, studies in *Vhl* knock-out mice indicates that other genetic and/or epigenetic

events are possibly required for development of ccRCC (Hsieh et al., 2017b; Wei and Hsieh, 2015). Large scale genomic studies have revealed the most frequently mutated genes apart from *VHL* in ccRCC: *PBRM1* (polybromo1), *SETD2* (SET domain containing 2) and *BAP1* (BRCA1 associated protein 1) (Cancer Genome Atlas Research Network, 2013; Hakimi et al., 2013a; Peña-Llopis et al., 2012; Sato et al., 2013). Specifically, PBRM1 is a component of PBAF SWI/SNF chromatin remodeling complex and has a role in preventing amplification of HIF oncogenic signals, SETD2 has important role in tumor cell lysine methylation of the histone H3 (Li et al., 2019), while BAP1 as a nuclear deubiquitinase is involved in the host cell factor 1 pathway and cell proliferation (Peña-Llopis et al., 2012). These genes represent three additional tumor suppressor genes, also located on chromosome 3p adjacent to *VHL*. It seems that 3p loss is frequent hallmark and represents early genetic event in ccRCC tumorigenesis, resulting in haploinsufficiency of four tumor suppressor genes (Hsieh et al., 2017b). Moreover, mutations of these genes have been associated with tumor progression and aggressive clinical features of ccRCC (Hakimi et al., 2013b; Nam et al., 2015).

The second most common chromosomal aberration in ccRCC is copy number gain of chromosome 5q, resulting in amplification of approximately 60 genes (Cancer Genome Atlas Research Network, 2013), including *EZH2*, *STC2*, *SQSTM1* and *VCAN* (Sanchez and Simon, 2018). These events affect complex processes, such as histone modification, stress response, mTOR regulation, and cell adhesion and migration (Haddad and Margulis, 2015). Also, it was demonstrated that several activating genomic alterations in the components of mTOR pathway might be involved in the progression of ccRCC (Cancer Genome Atlas Research Network, 2013; Sato et al., 2013).

1.1.4.2 Affected signaling pathways in ccRCC

The inactivation of *VHL* tumor suppressor gene, as the essential molecular event in RCC leading to HIF activation, promotes tyrosine kinase activity with consequent activation of RAS/MEK/ERK signaling pathway, phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt)/mTOR pathway and nuclear factor kappa B (NF-κB) pathway (Kumar et al., 2018). Namely, nuclear translocation of accumulated cytosolic HIFα leads to formation of active HIF through interaction with HIFβ and binding to hypoxia response element (HRE) that results in the activation of growth factors and

angiogenic proteins, among other hypoxia inducible genes. This is followed by overexpression of VEGF, which binds to its tyrosine kinase receptors (VEGF-R2) on both endothelial and ccRCC cells (Kumar et al., 2018). Dimerized receptors activate either RAS/MEK/ERK or PI3K/Akt/mTOR pathway heightening tumor progression by additional HIFα production (Sanchez and Simon, 2018), hence forming a positive feedback loop contributing to constitutive activation of the signaling network (Guo et al., 2015).

Altered genes of PTEN (phosphatase and tensin homologue deleted on chromosome 10) and mTOR signaling proteins have been identified as activators of other signaling pathways, like STAT (signal transducer and activator of transcription) and sonic hedgehog, contributing to RCC progression (Kumar et al., 2018).

Activated HIF also promotes binding of transforming growth factor alpha (TGFα) to its epidermal growth factor receptor (EGFR) that results in activation of PI3K/Akt/IκB-α/NF-κB signaling cascade (An and Rettig, 2005). The results by Zhou et al. suggest that interleukin-8 (IL‐8), highly expressed in metastatic RCC, induces the epithelial‐mesenchymal transition of RCC through the activation of the Akt signaling pathway. Namely, it seems that IL‐8promotes migration and invasion of RCC cells by elevation of phosphorylated Akt levels. Acquired tumor cell motility and invasiveness leads to enhanced metastatic ability. Induction and maintenance of epithelial‐mesenchymal transition as a step of tumor progression, enabled by activated Akt and various other signaling pathways may be a potential molecular mechanism for RCC metastasis (Zhou et al., 2016).

Beside activation of the PI3K/Akt pathway by extracellular signals and transmembrane receptors (Figure 1), multiple other mechanisms could contribute to its constitutive activation in ccRCC (Guo et al., 2015). Epigenetic regulatory mechanisms, such as interaction of non-coding RNAs, specifically microRNAs (miRNAs), with its target messenger RNA to inhibit protein translation, have recently emerged as important regulators of the PI3K/Akt pathway (Braga et al., 2019). Novel Akt activation mechanism by enhanced protein complex formation with phosphoinositide-dependent kinase-1 (PDK1) and 78-kDa glucose-regulated protein has been shown under glucose-deprivation conditions in multiple RCC cell lines (Guo et al., 2015).

All mentioned mechanisms imply the multifaceted nature of ccRCC at the molecular level.

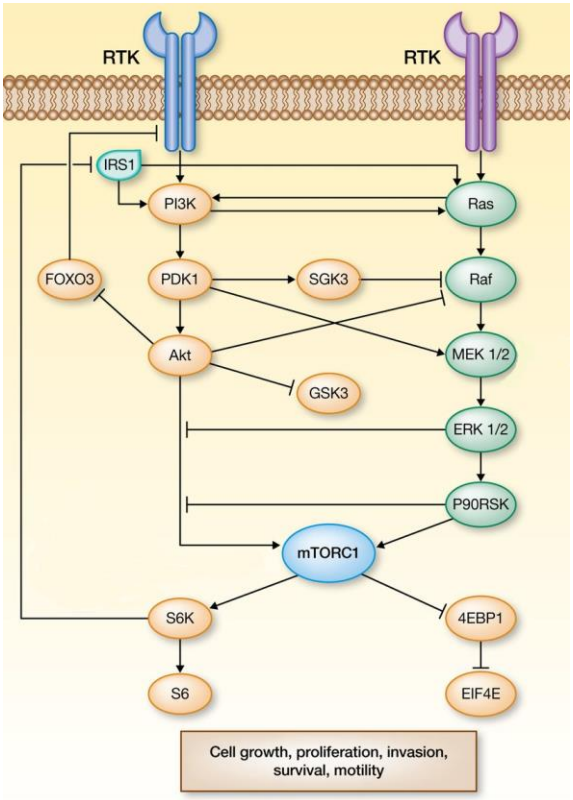

Figure 1. PI3K/Akt/mTOR and RAS/MEK/ERK signaling pathways mediate cell growth, proliferation, and invasion in cancer; Abbreviations: PDK1- phosphoinositide-dependent kinase-1, PI3K- phosphoinositide 3- kinase, P90RSK1: 90 kDa ribosomal protein S6 kinase 1, mTOR-mammalian target of rapamycin, S6K-ribosomal protein S6 kinase, ERK- extracellular signal-regulated kinase, RTK- tyrosine kinase receptor (Reproduced from Jahangiri and Weiss, 2013 (Jahangiri and Weiss, 2013), available at https://clincancerres.aacrjournals.org/content/19/21/5811)

1.1.4.3 Metabolic reprogramming in ccRCC

Numerous molecular mechanisms, both intrinsic and extrinsic by nature, alter cellular metabolic events, such as maintenance of energy status, enhanced macromolecules biosynthesis and tight control of redox homeostasis, in order to support basic needs in dividing cells (Cairns et al., 2011). Due to reduced blood supply, these

particular events are even more potentiated in cancer cells. In ccRCC, hypoxic conditions result in reprogramming of glucose, lipid and amino-acid metabolism, which makes this cancer a metabolic disease (Wettersten et al., 2015), characterized by glycogen and lipid accumulation in the cytoplasm of kidney cancer cells, hence, the "clear cell" type (Sanchez and Simon, 2018). Namely, up-regulation of glycolysis, lipid synthesis and tryptophane metabolism is accompanied with down-regulation of tricarboxylic acid (TCA) and urea cycle. In that way, the synthesis of cellular building blocks required for proliferation, together with high GSH/GSSG ratio enable the survival of tumor cells even in hypoxic nutritionally depleted environment (Wettersten et al., 2017). By modifying their metabolic phenotype, cancer cells maintain steady-state of high ROS (reactive oxygen species) levels within a narrow range, allowing them to increase growth and invasion and limit their apoptotic propensity (Laurent et al., 2005; Li et al., 2016).

1.1.5 Impaired redox homeostasis underlying ccRCC

It seems that in highly proliferative cancer cells, regulation of reactive oxygen species (ROS) production represents a crucial step. Aforementioned hypoxic conditions further promote ROS production, with consequential excess of downstream effects on signaling pathways and HIF1α accumulation (Cairns et al., 2011; Gao et al., 2013). Malignant cells respond to high ROS production by upregulation of antioxidant defense, apparently creating a certain paradox where high ROS steady-state levels are characterized by simultaneous increase of antioxidant levels. Indeed, cancer cells are able to become resistant to ROS by prompting a new redox steady-state (Sosa et al., 2013). Moreover, cancer cells release ROS into the tumor microenvironment affecting the adjoining cancer associated fibroblasts, consequently promoting stromal oxidative stress and autophagy. In this scenery, several crucial events are being initiated: a) angiogenesis - due to HIF1 activation and HIF-targeted signaling molecules (such as VEGF), as well as b) tumor growth and c) prevention of immune anti-cancer response facilitated by matrix metalloproteinases (MMPs), IL-6, IL-10, TGF, CCL2 and CCL5 (Sosa et al., 2013). Regarding the inflammatory aspect of RCC tumorigenesis, the serum levels of interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) seem to be increased in patients with RCC (Yoshida et al., 2002). Furthermore,

higher expression levels of IL-1β, IL-6¸ TNF-α, HIF-1α and MMP2 are associated with RCC cell lines with higher malignancy (Chuang et al., 2008). Moreover, the study by Petrella and Vincenti has shown that the pro-inflammatory cytokine IL-1β induced RCC tumor cell invasion (Petrella and Vincenti, 2012). The presence of tumor-associated macrophages and high serum levels of mentioned pro-inflammatory cytokines are poor prognostic factors in RCC patients, implying that an inflammatory microenvironment may promote RCC tumor progression (Petrella and Vincenti, 2012). Moreover, adjacent senescent cells can contribute by releasing pro-inflammatory cytokines and proteases into the tumor microenvironment, which farther promotes tumor growth and aggressiveness (Sosa et al., 2013). One way in which cancer cells respond to these damaging impacts of ROS is production of reduced glutathione (GSH), the key non-protein thiol antioxidant that efficiently neutralizes ROS -. Indeed, the high GSH content was found in RCC (Lusini et al., 2001). Recent study of Hakimi et al. demonstrated in late-stage ccRCC, high GSH content accompanied with its biosynthesis metabolites, such as cysteine and γ-glutamyl cysteine, (Hakimi et al., 2016). However, the decreased ratio of reduced and oxidized form of glutathione (GSH/GSSG), associated with lower activity of enzymes involved in GSH metabolism (glutathione peroxidase, glutathione transferase, γ-glutamyl transpeptidase, glutathione reductase) was demonstrated only in early-stage RCC (Lusini et al., 2001; Pljesa-Ercegovac et al., 2008). In summary, impaired redox homeostasis seems to be another significant hallmark of ccRCC. In support, nuclear factor (erythroid-derived 2)-like 2 (Nrf2) pathway, as the most important regulator of the redox homeostasis, is continually activated in ccRCC, primarily due to loss of Kelch-like ECH-associated protein 1 (Keap 1) function (Fabrizio et al., 2017). Since consequential nuclear accumulation of Nrf2 modifies the expression of numerous genes, including phase II detoxification and antioxidant enzymes, impairment of redox homeostasis in ccRCC might be attributed to changes in GSTs expression, as well(Tonelli et al., 2018).

1.2 Disease relevance of glutathione transferases

Glutathione transferases (GSTs) are multifunctional proteins known as phase II cellular detoxification system enzymes, yet, implicated in a number of catalytic and non-catalytic activities. Intracellularly, GSTs are strategically localized and perform

their functions in cytosol, as well as in mitochondria and microsomal portion of the cell. In particular, the cytosolic fraction is divided in seven classes, differing in chemical, physical and structural properties (Hayes et al., 2005). However, the functions of this family of enzymes, also termed as GSTome, may be classified into: (1) metabolism of xenobiotics and endogenous compounds (Hayes and Pulford, 1995), catalysis of crucial steps in the synthesis of leukotrienes, prostaglandins and steroid hormones, as well as the degradation of tyrosine (Board and Menon, 2013), and inactivation and reduction of oxidative stress by-products (Hayes and McLellan, 1999) and (2) the regulation of cell signaling pathways (including regulation of mitogen-activated protein kinases (MAPK) through protein-protein interactions) (Board and Menon, 2013; Tew and Townsend, 2012).

Their detoxification role of GSTs is based on conjugation reactions of wide variety of non-polar exogenous (carcinogens, environmental pollutants and anticancer drugs) and endogenous compounds with glutathione (GSH). In most cases, this conjunction yields more water-soluble products and therefore enables their elimination (Di Pietro et al., 2010; Hayes et al., 2005; Wu and Dong, 2012), however, this particular reaction can have an adverse effect, ensuing more reactive GSH-conjugate. Certain mutagens, carcinogens and pro-drugs are known to be metabolically bio-activated in this way (Figure 2) (Guengerich, 2005; Kurtovic et al., 2008).

Glutathione transferases show significance in terms of both development and progression of RCC (De Martino et al., 2010; Searchfield et al., 2011; Sweeney et al., 2000), not only by protecting cell vital macromolecules from variety of electrophilic compounds, however, by providing a certain antioxidant shield (Coles and Kadlubar, 2005; Hayes and McLellan, 1999). The role of GSTs in redox regulation emerged as important mechanism involved in cancer development and progression (Tew and Townsend, 2012). Namely, impaired redox homeostasis represents hallmark of ccRCC malignant phenotype. Specifically, ccRCC early phase is characterized by significant oxidative distress, followed by the presence of more reduced state in the course of ccRCC progression (Lusini et al., 2001). Lately, other significant functions of these enzymes have been recognized, including protein-protein interactions. Namely, GSTs act as modulators of the MAPK signaling pathway involved in cellular survival and apoptosis (Tew and Townsend, 2012). The ability of GSTs to participate in protein-

protein interactions with signaling molecules emphasizes the multiple signaling and regulatory functions of GSTs (Bartolini and Galli, 2016; Tew and Townsend, 2012).

Furthermore, polymorphisms in *GST* genes might affect cytosolic GST isoenzyme profile and consequently individual response to carcinogen exposure and pharmacogenomic-based cancer treatment (Lo and Ali-Osman, 2007). Genes encoding for cytosolic alpha (GSTA), mu (GSTM), pi (GSTP), theta (GSTT) members are highly polymorphic, due to either deletion or single nucleotide polymorphisms (SNPs). The effect of such prominent genetic heterogeneity on cancer propensity, as well as certain therapeutic consequences has been mostly studied considering aforementioned detoxification roles of GSTs. Hollman et al., even suggested a classification of diseases in relation to GST SNPs, including cancers (Hollman et al., 2016). Indeed, the association of common *GST* gene polymorphisms *(GSTM1*, *GSTT1*, *GSTP1* and *GSTA1)*, independently or in interaction with well-known risk factors, with higher propensity to RCC development has been shown (Coric et al., 2016; Zhong et al., 2018). Although the available results of both gene-gene and gene-environment studies are quite diverse, *GST* genotyping alone or in combination with other Phase I or Phase II gene polymorphisms could identify individuals that are at higher risk of developing RCC, especially those exposed to relevant substrates (Ahmad et al., 2012; Buzio et al., 2003; Coric et al., 2016; Karami et al., 2008; Yang et al., 2013).

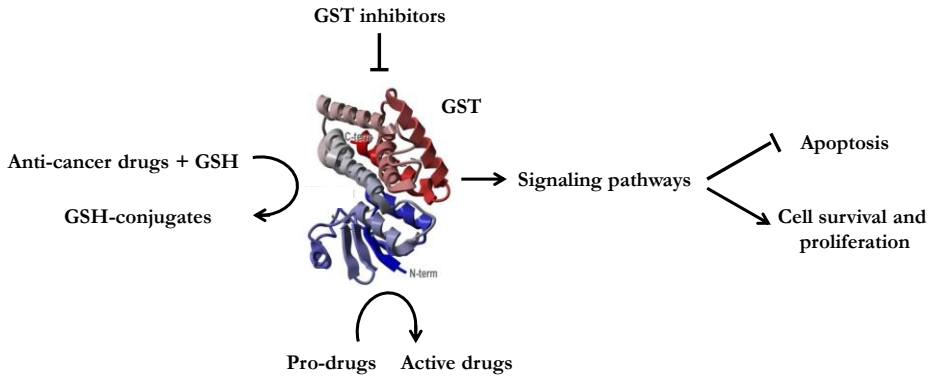

Figure 2. Different roles of glutathione transferases in chemoresistance. Apart from detoxification of conventional anti-cancer drugs, potential GSTs role in acquiring chemoresistance might also be mediated by modulating signaling pathways involved in cell proliferation and apoptosis. The novel drugs designed to selectively target GSTs comprise GST inhibitors or specific pro-drugs (Reproduced from Pljesa-Ercegovac et al. 2013, available at https://www.mdpi.com/1422-0067/19/12/3785)

1.3 Glutathione transferase omega class

Omega class GST, consisting of two isoenzymes GSTO1-1 and GSTO2-2, is one of the most recently characterized classes of cytosolic GSTs (Board et al., 2000). Members of this class are unique in many ways. First of all, they share approximately 20% amino acid sequence identity with members of the other classes (Board et al., 2000) and secondly, they possess cysteine residue in an active site, contrary to all other GSTs with catalytic tyrosine or serine residues (Whitbread et al., 2005). Additionally, GST omega class isoenzymes manifest the whole range of specific activities that are not associated with other human GSTs (Board and Menon, 2016). Namely, both enzymes are considered to be involved in the regulation of cellular redox homeostasis. GSTO2-2 is the enzyme with the highest dehydroascorbate-reductase (DHAR) activity that is responsible for preserving reduced form of ascorbic acid. On the other hand, GSTO1-1 contributes to cellular redox status by deglutathionylase activity (Board and Menon,

2016). Moreover, GSTO1-1 possesses several regulatory functions, including modulation of ryanodine receptors, activation of IL1-β and proposed anti-apoptotic role (Dulhunty et al., 2002; Laliberte et al., 2003; Piaggi et al., 2010). Additionally, it has been suggested that GSTO1-1 could also impact cancer chemoresistance by altering cell survival signaling pathways and inhibition of apoptotic MAPK signaling (Piaggi et al., 2010; Yan et al., 2007).

Despite their expression in a wide range of tissues GSTO1-1 and GSTO2-2 exhibit diverse tissue and cellular distribution. Relatively high GSTO1 expression was observed in the liver, heart, and skeletal muscle (Whitbread et al., 2005). The highest levels of GSTO2 mRNA have been shown in the testis, liver, kidney, and skeletal muscle (Whitbread et al., 2003). In several human cell types, such as macrophages, glial and endocrine cells, localization of human GSTO1 in the nucleus and nuclear membrane has been demonstrated using immunohistochemistry (Yin et al., 2001). This particular localization may indicate additional roles of GSTO1-1, unrelated to xenobiotic metabolism (Whitbread et al., 2005). Namely, nuclear translocation of GSTO1 might be involved in the neoplastic progression of *Barrett's* esophagus towards esophageal adenocarcinoma (Piaggi et al., 2009).

1.3.1 Glutathione transferase omega class polymorphisms

Two GSTO actively transcribed genes (*GSTO1* and *GSTO2)* were identified in the human population (Figure 3), located 1.5 kb apart on the long arm of chromosome 10 (10q25.1) (Board and Menon, 2016). Mukherjee et al. described a total of 31 polymorphisms in *GSTO1* and 66 polymorphisms of *GSTO2* gene (Mukherjee et al., 2006). Two commonly studied single nucleotide polymorphisms are: *GSTO1**C419A (rs4925) causing alanine to aspartate substitution in amino acid 140 (*Ala140Asp) and *GSTO2**A424G (rs156697) which causes an asparagine to aspartate substitution in amino acid 142 (*Asn142Asp). Additionally, recently several studies investigated a transition polymorphism in the position 183 at 5' untranslated region (5'UTR) of *GSTO2* gene(*GSTO2**A183G, rs2297235) (Wang et al., 2009). Strong linkage disequilibrium has been verified betweenthese three SNPs (Wang et al., 2009). Linkage disequilibrium represents the nonrandom association of alleles at different loci (Slatkin, 2008). Normalized coefficient of linkage disequilibrium (D') values can range from 0 to

1.0. Value of 1.0 indicates that two polymorphisms are maximally associated, whereas 0 indicates they are randomly associated (Hartl and Clark, 2007; Hedrick, 2011). Haplotype represents the combination of alleles on a single chromosome or, in a more restricted definition, all polymorphisms present on a single allele (The International HapMap Consortium, 2005).

It has been shown that *GSTO1* rs4925 polymorphism causes the change in abovementioned deglutathionylase activity, however, without influencing its monomethylarsonat reductase activity (Menon and Board, 2013; Tanaka-Kagawa et al., 2003; Whitbread et al., 2003). Regarding *GSTO2* rs156697 polymorphism, a strong association between variant *GSTO2*G* allele and lower *GSTO2* gene expression has been shown (Allen et al., 2012; Mukherjee et al., 2006). Consequently, SNPs of GST omega class enzymes have already been studied regarding numerous clinical disorders, such as Alzheimer's disease, Parkinson's disease, vascular dementia and stroke, amyotrophic lateral sclerosis, chronic obstructive pulmonary disease, as well as cholangiocarcinoma, hepatocellular carcinoma, breast and colorectal cancer (Board and Menon, 2016; Xu et al., 2014).

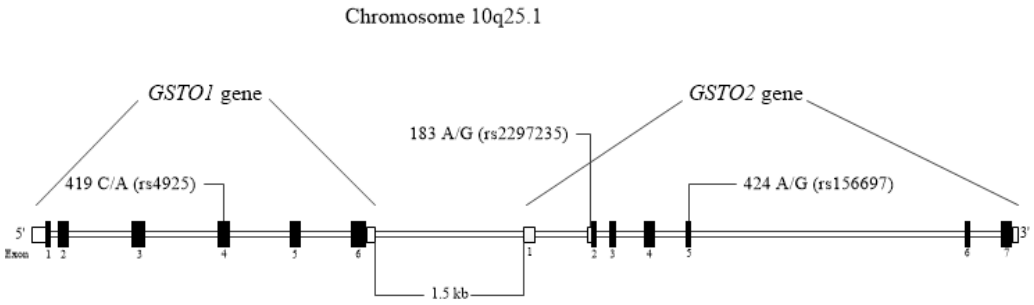

Figure 3. Schematic representation of the investigated polymorphisms in *GSTO1* and *GSTO2* genes. Exons that encode the open read frame - black rectangles; untranslated regions (UTRs)- white rectangles. (Reproduced from Radic et al. 2018, available at http://www.journal.med.tohoku.ac.jp/2461/246_35.pdf)

1.3.2 Structure of GSTO1 and GSTO2 enzymes

The members of the novel GST omega class, GSTO1 and GSTO2, have considerable structure similarity sharing approximately 64% of their amino acid

sequence. Both enzymes assemble as homodimers (GSTO1-1, GSTO2-2) (Figures 4 and 5) (Board et al., 2000; Whitbread et al., 2005). Human GSTO1 monomer is comprised of 241 amino acids and although its presumed size is 27.6kDa, it migrates on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) at approximately 31 kDa (Board et al., 2000). GSTO1-1 is comprised of thioredoxin-like N-terminal domain and a C-terminal domain that is composed completely of α-helices (Board et al., 2000). GSTO1 has some other specific features, such as proline-rich N-terminal extension of 19 amino acid residues which is not found in other GST family members (Whitbread et al., 2005). Human GSTO2 monomer is comprised of 243 amino acids (Whitbread et al., 2003). Owing to its high cysteine content (4.5%) and consequent difficulties in accomplishing purification, GSTO2-2 has not been studied in the same degree as GSTO1-1 (Board, 2011).

In contrast to other members of the GST family with active site tyrosine and serine residues, specific feature of GSTO1-1 and GSTO2-2 is an active site cysteine residue at position 32 (Cys-32). It has been shown that Cys-32 in GSTO1-1 forms disulfide bond with glutathione in the "G" site (Board et al., 2000). Based on data on the loss of thioltransferase activity caused by experimental Cys-32 mutation to alanine and the sensitivity of GSTO1-1 to alkylating agents (Board et al., 2000), it was concluded that the Cys-32 has important catalytic role in the thioltransferase reaction (Whitbread et al., 2005).

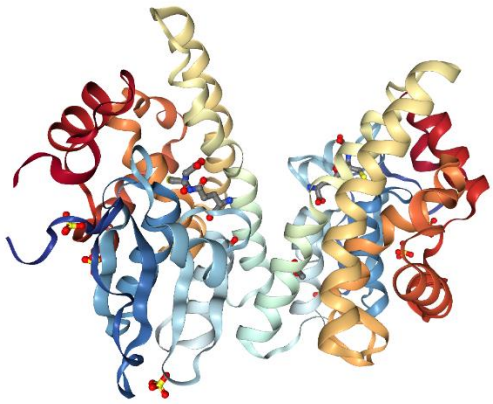

Figure 4. Structure of GSTO1-1 in complex with glutathione (Reproduced from RCSB protein databank, available at 10.2210/pdb5YVN/pdb)

Unlike other GSTs that possess highly hydrophobic "H" site for the binding of hydrophobic substrates, GSTO1-1 has relatively large and less hydrophobic "H" site (Board et al., 2000). This feature, together with the active site positioned in a wide crevice, suitable even for large substrates (Board et al., 2000), leads to the conclusion that the GSTO1-1 substrate does not have to be extremely hydrophobic and may even be a protein (Whitbread et al., 2005). GSTO2-2 with its narrower "H" site catalyzes reactions with smaller substrates compared to GSTO1-1 (Zhou et al., 2012).

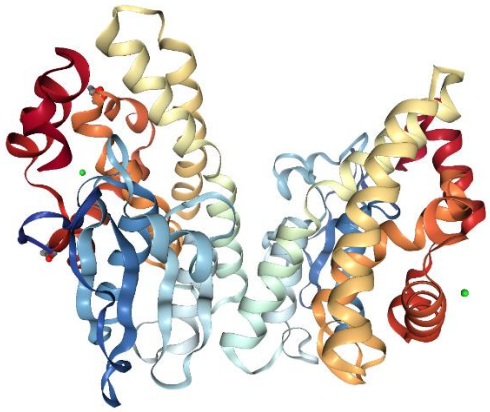

Figure 5. Structure of GSTO2-2 (Reproduced from RCSB protein databank, available at 10.2210/pdb3Q18/pdb)

1.3.3 Catalytic and regulatory roles of omega class glutathione transferases

Owing to the presence of cysteine residue in the active site, isoenzymes of omega GST class catalyze specific spectrum of glutathione-dependent thiol exchange and reduction reactions that are not associated with other cytosolic GSTs (Whitbread et al., 2005). Thioltransferase and dehydroascorbate reductase activities of GST omega class members are typical for glutaredoxins, enzymes showing structural similarity to the N-terminal domain of cytosolic GSTs (Whitbread et al., 2003). Although it was demonstrated that both isoenzymes catalyze reduction of dehydroascorbate, GSTO2-2 emerged as the enzyme with the highest activity in mammals (Schmuck et al., 2005). It has also been shown that both omega class isoenzymes play a significant role in arsenic biotransformation by catalytic reduction of monomethyl arsenate (Zakharyan et al., 2001). The reduction of S-(phenacyl) glutathiones to acetophenones has been associated

specifically with GSTO1-1 (Figure 6), but not with GSTO2-2 (Board and Anders, 2007). In contrast to GSTO2-2, GSTO1-1 plays important role in the glutathionylation cycle by its deglutathionylase and glutathionylase activity, depending on different conditions (Menon and Board, 2013). GSTO1-1 also exhibits numerous regulatory roles, including modulation of ryanodine receptors, activation of IL1-β and proposed anti-apoptotic role (Dulhunty et al., 2002; Laliberte et al., 2003; Piaggi et al., 2010).

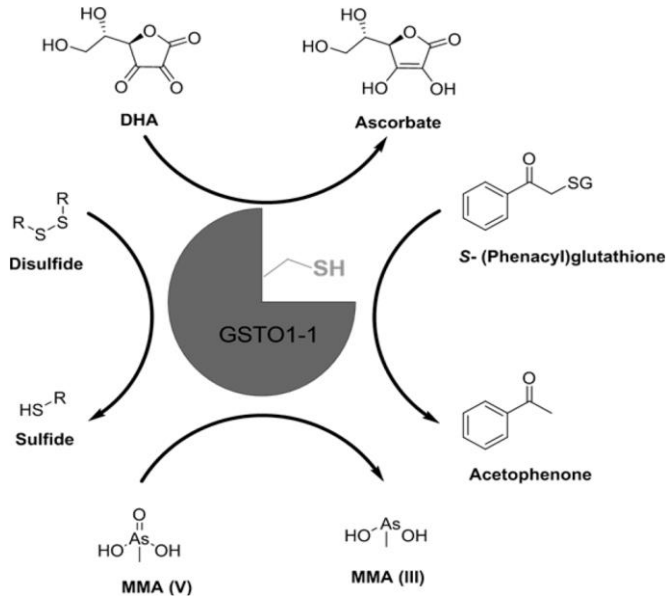

Figure 6. Dehydroascorbate reductase, monomethylarsonat reductase, thioltransferase, and S-(phenacyl) glutathione reductase activity of GSTO1-1. (Reproduced from Xie et al. 2018)

1.3.3.1 Deglutathionylase activity

Glutathionylation is formation of reversible disulfide bonds between protein thiols and glutathione. It has been shown that glutathionylation of intracellular protein thiols during oxidative stress contributes to efficient protection from irreversible oxidation (Cooper et al., 2011). Increased glutathionylation levels in response to oxidative stress (Board and Menon, 2013) can be reversed by deglutathionylase activity of glutaredoxins, thioredoxins and sulfiredoxin in the conditions of physiological redox homeostatis (Lei et al., 2008; Mieyal et al., 2008). Although defense against oxidative stress has been considered the main role of glutathionylation, it has been shown that it affects the functional and structural integrity of large group of proteins, known as the

"glutathionome" (Lindahl et al., 2011). The selective glutathionylation or deglutathionylation of specific protein thiols contributes to numerous cellular processes, such as cell cycle regulation, cytoskeleton remodeling, epigenetic DNA modifications, apoptosis, response to chemotherapy and the progression of neurodegeneration (Menon and Board, 2013). It was assumed for many years that novel omega class GSTs might be involved in the glutathionylation cycle, mainly based on significant structural similarity to glutaredoxin and the ability to accommodate large substrates. Indeed, the study by Menon and Board showed that GSTO1-1 plays significant role in the glutathionylation cycle catalyzing both the glutathionylation and deglutathionylation of proteins (Figure 7) (Menon and Board, 2013). It has been shown that GSTO1-1 exhibits specificity for particular proteins or particular glutathionylated cysteine residues (Board and Menon, 2016). Preliminary studies identified β-actin, heat shock protein 70, heat shock protein 7c and prolactin-inducible protein as specific targets for GSTO1-1-catalyzed deglutathionylation (Menon and Board, 2013). However, despite its great structural similarity to GSTO1-1 and the same active site cysteine residue at position 32 (Cys-32), GSTO2-2 did not exhibit the deglutathionylase activity (Menon and Board, 2013).

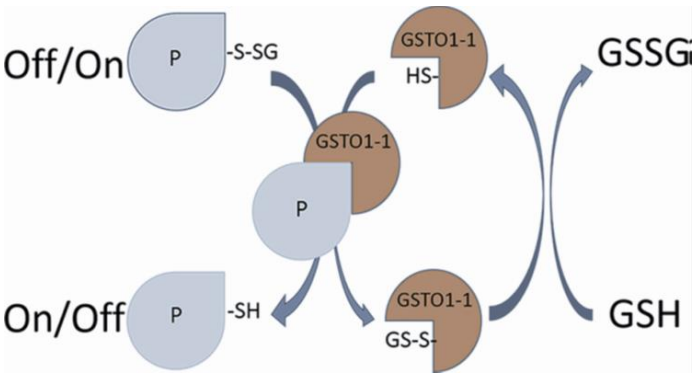

Figure 7. Potential on/off regulation of protein function resulting from specific deglutathionylation by GSTO1-1. (Reproduced from Xie *et al.* 2018)

1.3.3.2 Dehydroascorbate-reductase activity

GSTO1-1 and GSTO2-2 catalyze the reduction of dehydroascorbate to ascorbic acid (vitamin C) (Zhou et al., 2012), that plays an important role in the prevention of oxidative stress by scavenging reactive oxygen species (Frei et al., 1989). GSTO2-2 exhibits 70-100 times higher DHAR activity compared to GSTO1-1 and is considered to be the enzyme with the highest DHAR activity in mammalian cells (Schmuck et al., 2005). Thus, GSTO2-2 plays a key role in the maintenance of ascorbic acid, especially in the tissues in which ascorbic acid is actively transported in the form of dehydroascorbate, and subsequently enzymatically reduced to ascorbic acid in the cells (Zhou et al., 2012).

1.3.3.3 Arsenic biotransformation

Both GST omega class enzymes, GSTO1-1 and GSTO2-2, catalyze the reduction of pentavalent methylated arsenic species, monomethylarsenate^{V} (MMA^{V}) and dimethylarsenate^{V} (DMA^{V}) (Schmuck et al., 2005; Zakharyan et al., 2001). These reduction reactions are glutathione dependent (Whitbread et al., 2005). The recent studies' data on DMA^{V} reductase activity in rat liver cytosol and inhibition of the reaction by the GSTO1-1 inhibitor KT53 suggest that GSTO1-1 catalyzes the reduction of DMA^{V} in vivo (Németi et al., 2015). Although arsenic is a highly toxic and carcinogenic (Zakharyan and Aposhian, 1999), arsenic trioxide is used as a therapeutic treatment in patients with acute promyelocytic leukemia (Westervelt, 2001). Individual differences in response to therapy (Westervelt, 2001) suggest that genetic polymorphisms in the genes encoding the enzymes involved in arsenic biotransformation may be an important factor (Whitbread et al., 2005).

1.3.3.4 Regulation of post-translational modification of interleukin-1β

Interleukin 1β (IL-1β) is a proinflammatory cytokine produced by activated monocytes and macrophages and requires posttranslational processing before it is secreted. The active form of caspase-1 is necessary for the proteolytic cleavage of pro-IL-1β into 17 kDa active form. Caspase-1 activation is mediated by multi-protein complexes called inflammasomes. Laliberte et al. identified GSTO1-1 as a target of cytokine release inhibitory drugs (CRIDs) that blocks the release of active IL-1β and

assumed that the effect of those drugs is achieved by binding to GSTO1-1 in monocytes (Laliberte et al., 2003). Moreover, Coll and O'Neill concluded that GSTO1 may be a component of the inflammasome (Coll and O'Neill, 2011). Considering the inability of the active-site C32A GSTO1-1 mutant to bind CRID in the same manner as the wild type protein, the catalytic activity of GSTO1-1 might have the crucial role in IL-1β processing (Laliberte et al., 2003). GSTO1-1 could mediate an effect on IL-1β processing by its glutathionylase/deglutathionylase activity potentially modulating the function of a range of proteins in activated monocytes with altered redox homeostatis (Laliberte et al., 2003).

1.3.3.5 Ryanodine receptors modulation

Ryanodine receptors (RyRs) are homotetrameric proteins and the largest known ion channels that are encoded by three genes in mammals: RyR1 is the predominant isoform in skeletal muscle, RyR2 is the main isoform expressed in the heart, and RyR3 is expressed in other tissues (Dulhunty et al., 2011; Lanner et al., 2010). Ryanodine receptor intracellular Ca^{2+} release channels are located on the endoplasmic reticulum of smooth muscle cells and non-muscle cells and on the sarcoplasmic reticulum of striated muscle fibers. RyRs are responsible for the release of Ca^{2+} from the intracellular stores in response to a variety of intracellular and/or extracellular stimuli (Dulhunty et al., 2011).

GSTO1-1 has been shown to modulate RyRs. Specifically, GSTO1–1 inhibits cardiac RyR2 activity and potentiates skeletal muscle RyR1 activity (Dulhunty et al., 2011). Also, it has been shown that RyR2 channels with low activity were less affected by GSTO1-1 inhibition than channels with higher initial activity. It seems that one of the functions of GSTO1-1 in the heart is to decrease RyR2 activity during diastole to enable adequate filling of the sarcoplasmic reticulum Ca^{2+} store during diastole and to protect cardiac cells from high cytosolic Ca^{2+} concentrations that could trigger the delayed after depolarizations resulting in arrhythmia and sudden cardiac death (Dulhunty et al., 2001; Györke, 2009). Recently, Lu et al. showed that chemotherapy-induced GSTO1-1 expression, which is dependent on HIF-1 and HIF2, led to breast cancer stem cells enrichment. The underlying mechanism is GSTO1-1 interaction with

RyR1 and promotion of Ca^{2+} ion release from the endoplasmic reticulum and subsequent activation of PYK2/SRC/STAT3 signaling (Figure 8) (Lu et al., 2017).

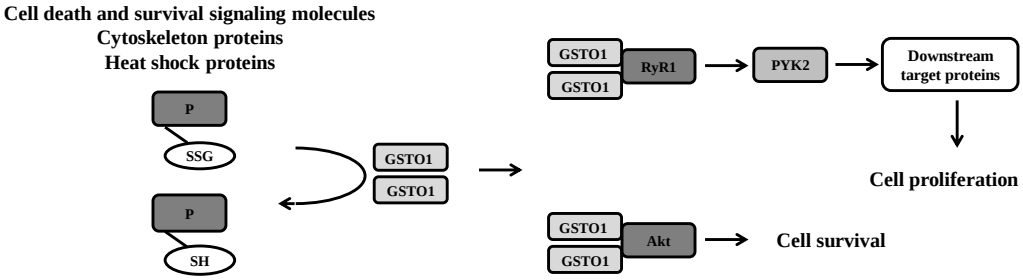

Figure 8. Proposed regulatory roles of GSTO1-1. Abbreviations: RyR1-ryanodine receptor type 1; PYK2- proline-rich tyrosine kinase 2; Akt-protein kinase B. (Reproduced from Pljesa-Ercegovac et al. 2018, available at https://www.mdpi.com/1422-0067/19/12/3785)

1.3.4 The relevance of omega class glutathione transferases in non-malignant and malignant diseases

1.3.4.1 Omega class glutathione transferases in non-malignant diseases

Until now, numerous studies have investigated the relevance of GSTO polymorphisms in regard to different non-malignant diseases. The study by Allen and colleagues investigated association of two commonly studied GST omega class polymorphisms *GSTO1* rs4925 (*Ala140Asp) and *GSTO2* rs156697 (*Asn142Asp) with disease risk and age-at-diagnosis of late-onset Alzheimer disease and Parkinson disease. They found significantly increased risk for late-onset Alzheimer disease in carriers of variant *GSTO2* allele and association of variant *GSTO1* allele with decreased risk in Parkinson disease. They also showed association of *GSTO1* and *GSTO2* variant alleles with lower *GSTO2* gene expression in the brain (Allen et al., 2012). Considering strong linkage disequilibrium between *GSTO1* and *GSTO2* SNPs (Wang et al., 2009) it is possible that another polymorphism located in the binding site of a transcription

factor that regulates the expression of *GSTO2* could be in linkage disequilibrium with investigated SNPs (Board and Menon, 2016). Kölsch *et al*. suggested that *GSTO1* rs4925 might modulate the severity and expansion of cerebrovascular atherosclerosis (Kölsch et al., 2007). Piacentini *et al.* found 4.56-fold increase in the risk of developing hypothyroidism in heterozygous carriers of variant *GSTO2**D142 (rs156697) allele (Piacentini et al., 2013). Several studies investigated GST omega polymorphisms in chronic obstructive pulmonary disease (COPD) finding association of *GSTO1* rs4925 and *GSTO2* rs156697 polymorphisms with low levels of the lung function parameters (Wilk et al., 2007). Yanbaeva *et al.* found increased risk of COPD in carriers of *GSTO1**140D/*GSTO2**142D haplotype (Yanbaeva et al., 2009). Also, high levels of GSTO1-1 have been demonstrated in alveolar macrophages of lung tissue from COPD patients (Harju et al., 2007). Stamenkovic *et al.* found association of variant *GSTO2**Asp (rs156697) allele with increased risk of age-related cataract development in smokers and individuals professionally exposed to ultraviolet irradiation (Stamenkovic et al., 2014).

1.3.4.2 Omega class glutathione transferases in cancer

Numerous studies have also been performed to investigate the role of GSTO polymorphisms in cancer susceptibility. The results by Djukic *et al*. indicate that *GSTO1**C (rs4925)/*GSTO2**G (rs156697) haplotype is associated with increased risk for development of transitional cell carcinoma (TCC) of urinary bladder. The modifying effect of *GSTO2* variant genotype on individual susceptibility to disease is more pronounced when associated with smoking (Djukic et al., 2015). Furthermore, mentioned GSTO polymorphisms were independent predictors of a higher risk of death among patients with muscle invasive bladder cancer (Djukic et al., 2013). Also, it has been shown that upregulated expression of GSTO1 in tumor tissue compared to non-tumor tissue correlates with TCC grade and stage (Djukic et al., 2017). Marahatta *et al.* have shown that *GSTO1* rs4925 polymorphism could be an important risk factor in susceptibility to hepatocellular carcinoma, cholangiocarcinoma, and breast cancer (Marahatta et al., 2006). However, a large meta-analysis by Xu *et al.* concluded that *GSTO1* polymorphism was not associated with cancer risk, in contrast to *GSTO2* polymorphism that was associated with higher breast cancer risk (Xu et al., 2014).

Regarding possible role of GST omega class enzymes in breast cancer Lu *et al.* showed HIF-dependent expression of GSTO1-1 in breast cancer cells exposed to carboplatin with consequent breast cancer stem cell enrichment. Additionally, they demonstrated that GSTO1-1 knockdown blocks cancer stem cell enrichment, tumor initiation, and metastasis (Lu et al., 2017). The overexpression of GSTO1-1 has been also reported in esophageal squamous cell carcinoma (Li et al., 2014), pancreatic cancer (Chen et al., 2009), and ovarian cancer (Yan et al., 2007). The study by Piaggi *et al.* showed that overexpression of GSTO1-1 following cisplatin treatment of HeLa cells seems to be associated with the activation of survival signaling pathways and inhibition of apoptotic MAPK pathway (Piaggi et al., 2010).

1.3.4.2.1 GSTO1-1 inhibitors: clinical perspectives

In the past few years, a diverse array of small molecules has been identified as GSTO1-1 inhibitors and have been previously developed regardless of GSTO1-1 activity. Specifically, this class of GSTs seems to be more susceptible to generic thiol-alkylating agents, due to presence of a functional cysteine residue in the catalytic center (Whitbread et al., 2005; Xie et al., 2018). Moreover, another class of highly specific as well as highly sensitive inhibitors of GSTO1 are α-chloroacetamide -1 group that react irreversibly with the active-site cysteine of this enzyme (e.g. ML175 and KT53) (Tsuboi et al., 2011, 2010), causing rapid inactivation of intracellular GSTO1-1. Moreover, in the human breast cancer cell line MDA-MB-435, KT53 caused a significant increase in cisplatin-induced cell death (Tsuboi et al., 2011). ML175, a specific GSTO1-1 inhibitor, is an activity-based inhibitor which covalently labels the active site cysteine nucleophile (Tsuboi et al., 2010). What is more, ML175 was shown to block lipopolysaccharide-stimulated inflammatory signaling which provided a field of possibility towards the development of novel anti-inflammatory drugs (Menon et al., 2014). So far, the most potent inhibitor of GSTO1-1 in the group of α-chloroacetamide compounds is proved to be C1-27 (Ramkumar et al., 2016), both providing covalent association with the active site cysteine (C32) and incorporating hydrophobic and hydrophilic interactions in the H-site. The bound C1-27 interacts predominately with residues in the H-site with only three interactions with the glutathione-binding site (G-site). C1-27 acts as a slow-turnover substrate demonstrated by 86% recovery of enzyme activity after pre-

incubation and a large dilution in the GSTO1 substrate assay. Indeed, C1-27 has proved to have promising antitumour activity in both *in vitro* and *in vivo* models of colorectal cancer, without gross systemic toxicities (Ramkumar et al., 2016). As far as irreversible inhibition of GSTO1-1 is concerned, Pace and co-workers reported that NJP2 (small peptide sulfonate ester) engages with enzyme by specific covalent modification of the active site cysteine, however, only within apoptotic cells (Pace et al., 2012). Another irreversible, yet selective inhibitor of GSTO1-1 is 5-chloromethylfluoresceindiacetate (CMFDA) reported by Son and colleagues (Son et al., 2010).

Considering recognized role of other GST classes in development and progression of RCC and intriguing range of both catalytic and non-catalytic roles of omega class GSTs, it could be beneficial to investigate role of GSTO1 and GSTO2 in the most aggressive RCC subtype (ccRCC). Therefore, this research for the first time investigated the modulating effect of three *GSTO1* and *GSTO2* gene polymorphisms, independently and in conjuction with recognized risk factors, on susceptibility to ccRCC. Furthermore, the prognostic role of these polymorphism was estimated. Additionally, expression profile of GSTO1-1 and GSTO2-2 proteins was determined, as well as phosphorylation status of specific downstream effectors of two pro-survival pathways, PI3K/Akt/mTOR and ERK-MAPK implicated in ccRCC. Moreover, possible association of GSTO1-1 with signaling molecules suggested to be regulated by glutathionylation was also investigated.

2 THE AIMS

The aim of this study was:

1. To evaluate the role of *GSTO1* (4925) and *GSTO2* (rs156697 and rs2297235) gene polymorphisms in susceptibility to development of clear cell renal cell carcinoma, individually and in interaction with established RCC factors (smoking, obesity and hypertension)
2. To evaluate the role of *GSTO1* (4925) and *GSTO2* (rs156697 and rs2297235) gene polymorphisms in prognosis of ccRCC patients
3. To evaluate the difference in expression of GSTO1-1 and GSTO2-2 enzymes in ccRCC tissue and corresponding non-tumor tissue
4. To evaluate the association of GSTO protein and specific signaling molecules of MAP kinase and phosphatidylinositol-3-OH kinase signaling pathways in ccRCC tissue

In addition to aforementioned aims, association of GST Omega gene variants with byproducts of oxidative DNA damage and correlation of GSTO1 protein expression with interleukin-1β activation in ccRCC tissue, as well as phosphorylation status of MAP kinase and phosphatidylinositol-3-OH kinase signaling molecules in ccRCC tissue were evaluated as additional objectives of this study.

3 MATERIALS AND METHODS

3.1 Study population

The case-control study comprising 239 subjects (162 men, 77 women; average age 58.94 ± 11.64 years) with histologically confirmed diagnosis of ccRCC and 350 sex- and age-matched controls (217 men, 133 women; average age 60.16 ± 11.11 years) was performed to investigate association of GSTO polymorphisms with ccRCC risk and prognosis. All ccRCC cases were recruited at the Clinic of Urology, Clinical Center of Serbia. Inclusion criteria for the ccRCC patients were: malignance established by ultrasonography, abdominal CT scan or MRI; confirmed histopathological diagnosis according to Eble et al. (Eble et al., 2006), modified by Srigley et al. (Srigley et al., 2013) and Tumor Node Metastais classification by Sobin et al. (Sobin et al., 2010); patients that have undergone partial or total nephrectomy; both genders, older than 18 years. The exclusion criterion was a preceding diagnosis of cancer.

The control group included subjects admitted to the same clinical center with benign conditions, unrelated to urological condition, excluding individuals with earlier cancer diagnosis. Controls included in this study were older than 18 years, both sexes with confirmed absence of malignance.

Structured questionnaire was used to acquire the data regarding basic demographic information, as well as information on established risk factors for ccRCC, such as obesity, smoking history and hypertension. In our study, obese patients were defined as individuals with BMI above 30kg/m^2. Smokers were defined as individuals who reported everyday smoking during a minimum of 60-day period prior to their enrollment in the study. All participants were questioned about the number of cigarettes smoked per day and duration of smoking. All gathered data referred to a time period prior to the diagnosis of ccRCC for the cases, and a corresponding period for the controls.

3.2 Ethics

The study was approved by the Institutional Ethical board (October 13th, 2011, approval number 29/X-3, Faculty of Medicine, University of Belgrade, Serbia and July 6th, 2017, approval number 29/VII-14). All protocols described here were performed in

strict accordance with ethical principles for medical research involving human subjects of the World Medical Association Declaration of Helsinki. Informed written consent was acquired from all participants.

3.3 Materials

Blood and tissue samples obtained from recruited subjects are part of the large bio-bank formed in collaboration of the Clinic of Urology, Clinical Center of Serbia, and the Institute of Medical and Clinical biochemistry, Faculty of Medicine.

3.3.1 Blood and plasma specimens

Whole blood samples were collected from subjects included in the study in vacutainer tubes with appropriate anticoagulant. 400µl of the sample was separated for deoxyribonucleic acid (DNA) isolation. Upon centrifugation at 3600 rpm/4°C plasma samples were obtained and stored at -80 °C.

3.3.2 Tissue specimens

Thirty tumor and corresponding non-tumor tissue samples were acquired from patients with ccRCC subjected to total nephrectomy. Histopathological examination was performed in all tissue samples to asses Fuhrman nuclear grade and stage of each tumor.

3.4 Methods

3.4.1 Genomic DNA isolation

Genomic DNA isolation was performed by *QIAamp DNA Blood Mini Kit* (*Qiagen, USA*). Blood samples were treated with detergent buffers and proteinase K to achieve lysis of samples and stabilization of DNA. Further, DNA adsorption onto the silica membrane of spin columns was performed by centrifugation. Following washing of the DNA bound to the membrane purified DNA was eluted in storage buffer and stored at -20^0C. Purity and concentration of DNA was determined by measuring absorbances at 230, 260, 280 and 320 nm using *GeneQuant pro* (*Biochrom, UK*) spectrophotometer.

3.4.2 Genotyping

*GSTO1**C419A (rs4925) (assay ID: C_11309430_30), *GSTO2**A424G (rs156697) (assay ID:C_3223136_1) and *GSTO2**A183G (rs2297235) (assay ID: C_3223142_1) genotypes were determined by quantitative polymerase chain reaction (qPCR), performed on *Mastercycler ep realplex (Eppendorf, Germany)* using *TaqMan SNP Genotyping assays* (*Thermo Fisher Scientific*, USA). PCR reaction was performed using 5µl of each DNA sample with 2.5 µl of *Maxima™ Hot start Master mix* (*Thermo Fisher Scientific*, USA), 0.25µl of appropriate *TaqMan SNP Genotyping assay* and 2.25µl of distilled water. The amplification reaction was comprised of 30 repeated cycles of three steps: denaturation (4 min at 94^0C), annealing (30s at 60^0C) and extension (45s at 72^0C). The reaction was monitored, and obtained results analyzed by *Mastercycler ep realplex software (Eppendorf, Germany)*.

3.4.3 Determination of 8-OHdG, IL-1β and pro- IL-1β levels by ELISA

The concentration of plasma 8-hydroxy-2′-deoxyguanosine (8-OHdG) was determined by competitive enzyme-linked immunosorbent assay (ELISA) method using the *OxiSelect Oxidative DNA Damage ELISA* kit *(Cell Biolabs, Inc., USA),* in accordance with the manufacturer's instructions. Plasma sample and standard (50µl) were added to an 8-OHdG/bovine serum albumin (BSA) conjugate coated microwell plate. Following 10 minutes incubation at room temperature, an anti-8-OHdG antibody was added and incubated for one hour. After washing, secondary antibody-enzyme conjugate was added and incubated for one hour at room temperature. Following multiple washings, addition of *Substrate Solution*, and termination of reaction by *Stop Solution*, the absorbance was measured at 450nm as the primary wave lenght on *5060-006 Micro Plate Reader (LKB, Austria)*. 8-OHdG sample concentration was determined by standard curve prepared from seven standard dilutions. The results were expressed as ng/ml.

The quantitative detection of interleukin-1β (IL-1β) in ccRCC cytosolic fractions was assessed by *Platinum ELISA* (enzyme-linked immunosorbent assay) kit *(Affimetrix, eBioscience, San Diego, California, USA)* according to the manufacturer's instructions. 50µl of each sample/standard was added to microwell plate coated with monoclonal antibody to human IL-1β. In order to bind to IL-1β captured by the first antibody, a

biotin-conjugated anti-human IL-1β antibody was added. After two-hour incubation and washing of unbound biotin-conjugated anti-human IL-1β antibody, streptavidin-horseradish peroxidase (HRP) was added. Following one-hour incubation and washing, 3, 3', 5, 5'-tetramethylbenzidine substrate solution was added to the wells. The reaction was terminated by addition of *Stop Solution* and absorbance was measured at 450nm on *5060-006 Micro Plate Reader (LKB, Austria)*. A standard curve was prepared from seven standard dilutions and IL-1β sample concentration determined and expressed as pg/ml.

The quantitative detection of pro-interleukin-1β (pro-IL-1β) in ccRCC cytosolic fractions was assessed by *Human pro-IL-1β ELISA* kit *(Elabscience Biotechnology Inc, Houston, Texas, USA)* according to the manufacturer's instructions. 100µl of each sample/standard was added to microwell plate coated with monoclonal antibody specific to human pro-IL-1β. After 90 minutes of incubation, a biotinylated detection antibody was added. After one-hour incubation and washing of unbound biotin-conjugated antibody, HRP conjugate was added. Following 30 minutes of incubation and washing, Substrate Reagent was added to the wells. The reaction was terminated after 15 minutes by addition of *Stop Solution* and absorbance was measured at 450nm on *5060-006 Micro Plate Reader (LKB, Austria)*. A standard curve was prepared from seven standard dilutions and pro-IL-1β sample concentration determined and expressed as pg/ml.

3.4.4 Identification of GSTO1, GSTO2, Akt and phosphorylated proteins of Akt/MAPK signaling pathway by Western blot

3.4.4.1 Tissue sample preparation

Cytosolic fractions of ccRCC tumor and corresponding non-tumor tissue samples (n=30) were obtained after homogenization in lysis buffer (50mmol/L Tris, 200mmol/L NaCl, 1mmol/L dithiothreitol, pH 7.8) supplemented with protease and phosphatase inhibitors (*Sigma-Aldrich, USA*). After two consecutive centrifugations at 3000rpm/4°C for 10 min and 36100rpm/4°C for 60 min, isolated cytosolic fractions were stored at -80 °C for further analysis.

3.4.4.2 Protein quantification

For the measuring of proteins concentration in cytosolic fractions *Bicinchoninic Acid Protein Assay* kit was used (*Sigma-Aldrich, USA*). The principle of the method is based on quantifying the reduction of the Cu^{+2} to Cu^{+1}, that is proportionate to sample protein concentration. Bovine serum albumin (BSA) protein standard curve was used for determining protein concentrations eventually expressed as g/l.

3.4.4.3 Western blot analysis

Acquired ccRCC and corresponding non-tumor cytosolic fractions were subjected to sodium dodecyl sulfate-polyacrilamide gel electrophoresis (SDS-PAGE) and Western blot for the expression of GSTO1, GSTO2, Akt, Akt (pT308), Akt (pS473), RSK1p90 (pS380), ERK1 (pY204)/ERK2 (pY187), RPS6 (pS235/236), Rab11a and β-tubulin by methods of Laemmli et al. (Laemmli, 1970) and Towbin et al. (Towbin et al., 1979).

Criterion™ TGX precast 26-well gels (4-15%) (*Bio-Rad, USA*) were used for electrophoresis of samples, each containing 30μg of total protein. Reduction of disulfide bonds and denaturation of proteins in sample was accomplished in loading buffer, comprising 2x Laemmli buffer (*Biorad, USA*) and 50mM dithiothreitol (DTT, *SERVA Electrophoresis GmbH, Germany*). The sample denaturation was performed at 95^0C for 5 minutes in *ThermoMixer C (Eppendorf, Germany)*. For the purpose of determining the protein size *PageRuler™ Prestained Protein Ladder (Thermo Fisher Scientific, USA*), ranging from 10 to 170 kDa was used.

Electrophoresis at 150V constant (4°C) was performed using *Bio-Rad Criterion™ Cell* (*Bio-Rad, USA*). *Bio-Rad Criterion™ blotter system (Bio-Rad, USA)* was used for transfer of proteins onto nitrocellulose membrane (100V constant, 4°C). Detection of proteins transferred to membrane was performed by using primary antibodies against GSTO1 (mouse polyclonal *Abcam, Cambridge, UK*), GSTO2 (rabbit polyclonal, *GeneTex, USA*), Akt (*Cell Signaling, USA*), phospho-Akt (T308) (rabbit monoclonal, *Cell Signaling, USA*) and β-tubulin (mouse monoclonal, *Sigma-Aldrich, USA*) diluted in 0.05% Tween20 Tris (*Sigma-Aldrich, USA*) according to the manufacturer's recommendations. *Akt/MAPK Signaling Pathway Antibody Cocktail* of 5 primary rabbit antibodies (1:500, *Abcam, UK*) was used for simultaneous detection of

phosphorylated 90kDa ribosomal protein S6 kinase 1 (RSK1p90) phospho-S380, protein kinase B (Akt) phospho-S473, extracellular-signal-regulated kinase (ERK1 phospho-Y204)/ERK2 phospho-Y187), ribosomal protein S6 (RPS6) phospho-S235/236 and Rab11a, as loading control protein. The cocktail targets downstream effectors of two important pro-survival pathways: the PI3K/Akt/mTOR and the Raf/MEK/ERK pathway. PI3K downstream effectors targeted in this cocktail are AKT1 phospho S473 and RPS6 phospho S235/236, whereas the downstream effectors of the MEK pathway are ERK1/2 phospho Y204/197 and p90RSK phospho S380. These two pathways are known for promoting cell growth, regulating apoptosis, chemotherapeutic drug resistance and cellular senescence. Further, membranes were incubated with appropriate HRP-conjugated secondary antibodies (1:8000, anti-mouse developed in goat, *Abcam, UK;* 1:3000, anti-rabbit developed in donkey, *GE Healthcare, UK).* Finally, the membranes were treated with *Clarity™ Western ECL Substrate (Bio-Rad, USA)* followed by detection of chemiluminescence on *ChemiDoc™ MP Imaging System (Bio-Rad, USA)*. Densitometry analysis of obtained blots was performed using *ImageLab software (Bio-Rad, USA)*.

3.4.5 Immunoprecipitation

Immunoprecipitation was performed using *Catch and Release® v2.0 High Throughput (HT) Immunoprecipitation* Assay *Kit* (*Merck Millipore, Germany*) in accordance with the manufacturer's protocol. ccRCC cytosolic fractions, set to protein concentration of 1µg/µl, were incubated with the mouse monoclonal anti-GSTO1 antibody (Abcam, *UK*) on filter microplate followed by the resuspension in *2xLaemlli buffer* (*Bio-rad, USA*), denaturated at 90°C/5 min and collected by centrifugation. Supernatant fraction containing immunoprecipitated proteins was subjected to electrophoresis andimmunoblot analysis in order to investigate potential association of Akt/MAPK signaling pathway proteins with GSTO1.

3.4.6 Statistical analysis

Statistical Package for the Social Sciences (*SPSS* software version 17, *SPSS Inc, USA*) was used for statistical analysis. Continuous variables were expressed as mean ± standard deviation (SD) or median (minimum-maximum), depending on data

distribution. Distribution was tested by using *Shapiro-Wilk* and *Kolmogorov–Smirnov* tests, as well as graphical methods. Categorical variables were presented as frequency (n, %) counts. Comparison of investigated variables was performed by *Student's* t test for continuous normally distributed variables and *Mann–Whitney* test for continuous variables with non-normal distribution. Comparison of categorical variables was performed by $\chi 2$ test. $\chi 2$ test was also used to test deviation of the genotype distribution from *Hardy-Weinberg* equilibrium for each polymorphism, in the patients and the controls individually.

The effect of GSTO genotypes on ccRCC risk was evaluated by logistic regression analysis and expressed by odds ratios (OR) and 95% confidence intervals (CI). OR was adjusted by age, gender, as well as by variables representing established risk factors for ccRCC: smoking status, hypertension and obesity. Assessment of linkage disequilibrium (LD) between SNPs and haplotype analysis were analyzed by the *SNPStats* (Solé et al., 2006). The LD strength was expressed as $D' = D/Dmax$.

The effect of GSTO genotypes on overall survival of ccRCC patients was evaluated by *Kaplan-Meier* analysis. Survival time was calculated as time from nephrectomy to the date of death or last follow-up (March 1st, 2018.). The follow-up data were available in 228 ccRCC patients due to the loss of 11 patients' contact information. Median follow-up was 67 months, ranging from 1 to 153 months. The long-rank test was used for the estimation of differences in survival according to the different genotypes of each SNP.

The prognostic value of three GSTO polymorphisms in overall mortality was evaluated by the *Cox* regression analysis, adjusted by Fuhrman nuclear grade and pT stage, as recognized prognostic factors.

The difference in expression of GSTO proteins in tumor compared to corresponding non-tumor tissue was evaluated by *Wilcoxon* test, while protein expression stratified according to pT stage and Fuhrman nuclear grade was analyzed by *Mann–Whitney* rank-sum test and *Kruskal-Wallis* test, respectively. The association between GSTO1 protein expression and IL-1β/pro-IL1β ratio was analyzed using Spearman's coefficient of linear correlation.

P value of ≤ 0.05 was considered to be statistically significant.

4 RESULTS

4.1 Relevance of glutathione transferase omega class gene polymorphisms in the development of clear cell renal cell carcinoma (ccRCC)

Gene polymorphisms of glutathione transferase omega class, *GSTO1* (rs4925) and *GSTO2* (rs156697 and rs2297235) were assessed in 239 patients with ccRCC and 350 controls.

4.1.1 Demographic and clinical characteristics of ccRCC patients and controls

Demographic and clinical characteristics of 239 patients with ccRCC and 350 controls are presented in Table 1. Average age of ccRCC group comprising 162 men and 77 women was 58.94 ± 11.64 years, whereas in control group which includes 217 men and 133 women, average age was 60.16 ± 11.11 years. Apart from age and gender, smoking status, hypertension and obesity were also included as established ccRCC risk factors. There was no significant difference between patients and controls with regards to age, gender and obesity ($p>0.05$). However, we found that 55% of ccRCC patients had hypertension, in contrast to 29% hypertensive controls. Furthermore, logistic regression analysis showed that hypertensive subjects were at 3.54-fold higher risk of ccRCC development compared to normotensive subjects (95%CI:2.35-5.32, $p<0.001$). Additionally, smokers exhibited 1.5-fold increased risk of ccRCC without reaching statistical significance (95%CI:0.99-2.26, $p=0.057$). As presented in Table 1, tumor grade II was shown to be the most frequent among ccRCC patients (G2, 55%). Regarding pT stage, the majority of patients had pT1 and pT3 tumors (45% and 42%, respectively).

Table 1. Demographic and clinical characteristics of ccRCC patients and controls

	Controls	Patients	OR (95% CI)	p
Age (years)[a]	60.16 ± 11.11	58.94 ± 11.64		0.207
Gender, *n* (%)				
Male	217 (62)	162 (68)	1.00	
Female	133 (38)	77 (32)	1.20 (0.79-1.84)[b]	0.391
Smoking, *n* (%)				
Never	164 (49)	80 (41)	1.00	
Ever[c]	173 (51)	114 (59)	1.50 (0.99-2.26)[d]	0.057
Pack-years[e]	30.00 (1.00-120.00)	31.25 (0.30-141.00)		0.267
Hypertension, *n* (%)				
No	232 (71)	89 (45)	1.00	
Yes	96 (29)	109 (55)	3.54 (2.35-5.32)[f]	<0.001
Obesity, *n* (%)				
BMI<30	253 (83)	157 (80)	1.00	
BMI≥30 [g]	50 (17)	39 (20)	1.09 (0.66-1.81)[h]	0.732
BMI (kg/m2)[a]	26.51 ± 3.83	26.65 ± 4.41		0.710
Fuhrman nuclear grade[i]				
G1		30 (15)		
G2		112 (55)		
G3		52 (26)		
G4		8 (4)		
pT stage[i]				
pT1		100 (45)		
pT2		24 (11)		
pT3		94 (42)		
pT4		5 (2)		

[a]mean ± SD; [b]OR, odds ratio adjusted to age, smoking status, hypertension, obesity; [c]Every-day smoking during a minimum of 60-day period prior to the study onset; [d]OR, odds ratio adjusted to age, gender, hypertension, obesity; [e]Median (min-max); [f]OR, odds ratio adjusted to age, gender, smoking status, obesity; [g]BMI, body mass index; Obese participants were defined as individuals with BMI above 30; [h]OR, odds ratio adjusted to age, gender, smoking status, hypertension; CI, confidence interval; [i]Available data on patients' tumor grade and stage, depending on the type of surgery and histopathology diagnostics; $p<0.05$ was considered to be statistically significant

4.1.2 The distribution of *GSTO1* and *GSTO2* genotypes in relation to ccRCC risk

Distribution of the *GSTO1* (rs4925) and *GSTO2* (rs156697 and rs2297235) genotypes in ccRCC patients and controls is presented in Table 2. The frequency of variant *GSTO1**A/A (rs4925) genotype was 12% in control group and 13% in ccRCC group, whereas variant *GSTO2**G/G (rs156697) genotype was present in 11% of controls and 12% of patients. Interestingly, variant *GSTO2**G/G (rs2297235) genotype was more frequent in control group. As indicated, carriers of variant *GSTO1**A/A (rs4925) and *GSTO2**G/G (rs156697) genotypes were at higher risk of ccRCC development when compared to referent *GSTO1**C/C (rs4925) and *GSTO2**A/A

(rs156697) genotypes, however this association did not reach statistical significance (OR=1.35, 95%CI:0.70-2.61, p=0.364 and OR=1.78, 95%CI:0.91-3.50, p=0.092, respectively).

Table 2. Distribution of *GSTO1*(rs4925) and *GSTO2* (rs156697 and rs2297235) genotypes in ccRCC patients and controls

Genotype	Controls, *n*(%)	Patients, *n*(%)	OR (95% CI)[a]	p
***GSTO1* rs4925[b]**				
*C/C	128 (38)	89 (38)	1.00	
*C/A	169 (50)	116 (49)	0.87 (0.56-1.33)	0.512
*A/A	41 (12)	31 (13)	1.35 (0.70-2.61)	0.364
***GSTO2* rs156697[c]**				
*A/A	149 (45)	92 (38)	1.00	
*A/G	148 (44)	119 (50)	1.26 (0.83-1.92)	0.283
*G/G	36 (11)	28 (12)	1.78 (0.91-3.50)	0.092
***GSTO2* rs2297235[d]**				
*A/A	163 (48)	97 (42)	1.00	
*A/G	133 (39)	111 (48)	1.27 (0.84-1.94)	0.263
*G/G	42 (12)	23 (10)	1.06 (0.54-2.06)	0.871

[a]OR, odds ratio adjusted to age, gender, smoking status, hypertension, obesity; CI, confidence interval; [b]For *GSTO1* rs4925, genotyping was efficient in 99% of patients and 97% of controls; [c]For *GSTO2* rs156697, genotyping was efficient in all recruited patients and 95% of controls; [d]For *GSTO2* rs2297235, genotyping was efficient in 97% of patients and 97% of controls; $p<0.05$ was considered to be statistically significant

However, when *GSTO1* rs4925 and *GSTO2* rs156697 polymorphisms were analyzed in combination, the significant association was obtained. Namely, ccRCC patients carriers of combined variant *GSTO1**A/A (rs4925) and *GSTO2**G/G (rs156697) genotypes showed 2.6-fold higher risk of cancer development in comparison with carriers of wild-type genotype combination (*GSTO1**C/C and *GSTO2**A/A) (95%CI:1.09-6.19, p=0.031). Moreover, combined effect of all three GSTO polymorphic variants showed no further increase in ccRCC risk (Table 3).

Table 3. Distribution of combined *GSTO1* (rs4925) and *GSTO2* (rs156697 and rs2297235) genotypes in ccRCC patients and controls

Genotype	Controls, *n*(%)	Patients, *n*(%)	OR (95% CI)[a]	p
Combined *GSTO1* rs4925/ *GSTO2* rs156697				
*CC+*CA/ *AA+*AG	267 (82)	197 (84)	1.00	
*CC+*CA/ *GG	17 (5)	8 (3)	0.77 (0.29-2.04)	0.602
*AA/ *AA+*AG	22 (7)	12 (5)	0.85 (0.37-1.97)	0.709
*AA / *GG	18 (6)	19 (8)	2.60 (1.09-6.19)	0.031
Combined *GSTO1* rs4925/ *GSTO2* rs2297235				
*CC+*CA/ *AA+*AG	277 (85)	195 (85)	1.00	
*CC+*CA/ *GG	9 (3)	3 (1)	0.41 (0.10-1.63)	0.205
*AA/ *AA+*AG	10 (3)	11 (5)	1.75 (0.63-4.89)	0.283
*AA / *GG	31(9)	20 (9)	1.27 (0.62-2.59)	0.517
Combined *GSTO2* rs156697/ *GSTO2* rs2297235				
*AA+*AG / *AA+*AG	276 (85)	200 (86)	1.00	
*AA+*AG / *GG	14 (4)	4 (2)	0.39 (0.12-1.27)	0.118
*GG / *AA+*AG	11 (3)	8 (4)	1.20(0.42-3.45)	0.741
*GG / *GG	25 (8)	19 (8)	1.55 (0.72-3.37)	0.264
Combined *GSTO1* rs4925/ *GSTO2* rs156697/*GSTO2* rs2297235				
*CC+*CA/ *AA+*AG/ *AA+*AG	259 (81)	187 (82)	1.00	
*AA / *GG/ *GG	18 (6)	19 (8)	2.57 (1.08-6.10)	0.033

[a]OR, odds ratio adjusted to age, gender, smoking status, hypertension, obesity; CI, confidence interval; $p<0.05$ was considered to be statistically significant

4.1.3 The association of *GSTO1/GSTO2* haplotype with the ccRCC risk

Our results on combined effects of GSTO polymorphisms were also confirmed by haplotype analysis. Namely, since both *GSTO1* and *GSTO2* genes are located on the same chromosome, just 1.5 kb apart we estimated the linkage disequilibrium (LD) between GSTO polymorphisms. Namely, we evaluated the nonrandom association of GSTO alleles and expressed it as normalized coefficient of LD (D'). Since D' values can range from 0 to 1.0, value of 1.0 indicates that two polymorphisms are maximally associated, whereas 0 indicates they are randomly associated (Hartl and Clark, 2007; Hedrick, 2011). We found a D' of 0.64 between *GSTO1* rs4925 and *GSTO2* rs156697 ($p<0.001$), 0.83 for *GSTO1* rs4925 and *GSTO2* rs2297235 ($p<0.001$) and 0.80 between *GSTO2* rs156697 and *GSTO2* rs2297235 ($p<0.001$), confirming a high LD between these pairs of SNPs. As indicated in Table 4, the most prevalent haplotype among controls (52%) and patients (56%) is H1, consisting of *GSTO1**C, *GSTO2**A (rs156697) and *GSTO2**A (rs2297235) wild-type alleles. The second most frequent is H2 haplotype comprised of all three variant alleles, *GSTO1**A (rs4925), *GSTO2**G (rs156697) and *GSTO2**G (rs2297235). Haplotypes H5 and H6 had the lowest frequencies in both patients and controls. We found that carriers of H2 haplotype, exhibited the highest risk of ccRCC development (OR=1.46, 95%CI:1.02-2.09, p=0.041) (Table 4).

Table 4. Haplotypes of *GSTO1* (rs4925) and *GSTO2* (rs156697 and rs2297235) in relation to the risk of ccRCC

Haplotype	*GSTO1* rs4925	*GSTO2* rs156697	*GSTO2* rs2297235	Controls, %	Patients, %	OR (95% CI)[a]	p
H1	*C	*A	*A	52	56	1.00	
H2	*A	*G	*G	22	31	1.46 (1.02-2.09)	0.041
H3	*A	*A	*A	9	5	0.55 (0.31-0.99)	0.047
H4	*C	*G	*A	6	5	0.95 (0.49-1.86)	0.880
H5	*A	*A	*G	5	1	0.23 (0.08-0.68)	0.008
H6	*C	*G	*G	4	1	0.06 (0.01-0.72)	0.027

Global haplotype association p-value: <0.0001; [a]OR, odds ratio adjusted to age, gender, smoking status, hypertension, obesity; CI, confidence interval; $p<0.05$ was considered to be statistically significant

4.1.4 Modulating effect of *GSTO1* and *GSTO2* genotypes in conjunction with established risk factors on ccRCC susceptibility

We also investigated possible modulating effect of GSTO genotypes in conjunction with hypertension, obesity and smoking, as established risk factors, on ccRCC risk (Tables 5-7). We found no modifying effect of GSTO genotypes with hypertension (Table 5) and obesity (Table 6). Hypertensive subjects were at significantly higher risk of ccRCC development regardless of *GSTO1* and *GSTO2* genotypes. Although obese carriers of variant *GSTO1**A/A (rs4925) and *GSTO2**G/G (rs2297235) genotypes exhibited higher ccRCC risk, the association was not statistically significant (OR=3.74, 95%CI:0.65-21.60, p=0.140; OR=4.05, 95%CI: 0.42-39.57, p=0.229). However, statistically significant modulating effect on ccRCC risk conferred by smoking has been found only in *GSTO2**G/G (rs156697) carriers (OR=2.44, 95%CI:1.04-5.71, p=0.040), whereas another two studied polymorphisms (*GSTO1* rs4925 and *GSTO2* rs2297235) did not show significant association with smoking (Table 7).

Table 5. Distribution of *GSTO1* (rs4925) and *GSTO2* (rs156697, rs2297235) genotypes in relation to hypertension in ccRCC patients and controls

	Controls, *n*(%)	**Patients, *n*(%)**	**OR (95% CI)[a]**	**p**
Combined *GSTO1* rs4925/ hypertension				
*CC+*CA/no	194 (61)	71 (36)	1.00	
*CC+*CA/yes	86 (27)	98 (50)	3.96 (2.54-6.16)	<0.001
*AA/no	30 (9)	15 (8)	1.95 (0.94-4.07)	0.074
*AA /yes	9 (3)	11 (6)	3.61 (1.34-9.67)	0.011
Combined *GSTO2* rs156697/ hypertension				
*AA+*AG /no	196 (62)	75 (38)	1.00	
*AA+*AG /yes	86 (27)	99 (50)	3.83 (2.47-5.93)	<0.001
*GG/no	25 (8)	14 (7)	2.10 (0.98-4.52)	0.057
*GG/yes	8 (3)	10 (5)	3.63 (1.28-10.26)	0.015
Combined *GSTO2* rs2297235/ hypertension				
*AA+*AG/no	196 (61)	75 (39)	1.00	
*AA+*AG/yes	83 (26)	97 (51)	3.80 (2.45-5.89)	<0.001
*GG/no	30 (10)	10 (5)	1.29 (0.57-2.91)	0.543
*GG/yes	11 (3)	10 (5)	2.43 (0.93-6.29)	0.069

[a]OR, odds ratio adjusted to age, gender, smoking status, obesity; CI, confidence interval; $p<0.05$ was considered to be statistically significant

Table 6. Distribution of *GSTO1* (rs4925) and *GSTO2* (rs156697, rs2297235) genotypes in relation to obesity in ccRCC patients and controls

	Controls, *n*(%)	**Patients, *n*(%)**	**OR (95% CI)[a]**	**p**
Combined *GSTO1*/ obesity[b]				
*CC+*CA/non-obese	213 (73)	134 (69)	1.00	
*CC+*CA/obese	48 (16)	34 (18)	1.02 (0.60-1.74)	0.948
*AA/ non-obese	29 (10)	20 (10)	1.31 (0.68-2.52)	0.413
*AA / obese	2 (1)	5 (3)	3.74 (0.65-21.60)	0.140
Combined *GSTO2* rs156697/ obesity				
*AA+*AG /non-obese	216 (75)	136 (69)	1.00	
*AA+*AG /obese	46 (16)	37 (19)	1.14 (0.67-1.93)	0.624
*GG/ non-obese	25 (9)	21 (11)	1.67 (0.86-3.25)	0.130
*GG/obese	3 (1)	2 (1)	1.00 (0.13-7.87)	0.997
Combined *GSTO2* rs2297235/ obesity				
*AA+*AG /non-obese	214 (72)	136 (72)	1.00	
*AA+*AG /obese	49 (16)	34 (18)	0.94 (0.56-1.61)	0.831
*GG/non-obese	32 (11)	15 (8)	0.79 (0.40-1.56)	0.489
*GG/obese	1 (1)	4 (2)	4.05 (0.42-39.57)	0.229

[a]OR, odds ratio adjusted to age, gender, smoking status, hypertension; CI, confidence interval; [b]Obese participants were defined as individuals with BMI (body mass index) above 30; $p<0.05$ was considered to be statistically significant

Table 7. Distribution of *GSTO1* (rs4925) and *GSTO2* (rs156697, rs2297235) genotypes in relation to smoking status in ccRCC patients and controls

	Controls, *n*(%)	**Patients, *n*(%)**	**OR (95% CI)[a]**	**p**
Combined *GSTO1* rs4925/ smoking[b]				
*CC+*CA/ non-smokers	140 (43)	67 (35)	1.00	
*CC+*CA/smokers	149 (46)	97 (51)	1.55 (0.99-2.42)	0.053
*AA/ non-smokers	17 (5)	12 (6)	1.91 (0.74-4.90)	0.182
*AA /smokers	20 (6)	15 (8)	1.91 (0.85-4.30)	0.116
Combined *GSTO2* rs156697/ smoking				
*AA+*AG / non-smokers	142 (44)	70 (36)	1.00	
*AA+*AG / smokers	151 (46)	99 (51)	1.51 (0.97-2.35)	0.067
*GG/ non-smokers	16 (5)	9 (5)	1.46 (0.54-3.97)	0.460
*GG/smokers	15 (5)	15 (8)	2.44 (1.04-5.71)	0.040
Combined *GSTO2* rs2297235/ smoking				
*AA+*AG/ non-smokers	138 (42)	71 (37)	1.00	
*AA+*AG/ smokers	153 (47)	100 (52)	1.45 (0.94-2.25)	0.095
*GG/ non-smokers	21 (6)	8 (4)	0.86 (0.32-2.31)	0.762
*GG/smokers	17 (5)	12 (6)	1.40 (0.59-3.33)	0.447

[a]OR, odds ratio adjusted to age, gender, hypertension, obesity; CI, confidence interval; [b]Smoking status was categorized into non-smokers and smokers with respect to the limit of a minimum of 60-day period of every-day smoking prior to their enrollment in the study; $p<0.05$ was considered to be statistically significant

4.1.5 The association of *GSTO1* and *GSTO2* genotypes with the plasma concentration of 8-OHdG in ccRCC patients

Considering antioxidant role of GSTO1 and GSTO2, we investigated the degree of oxidative DNA damage in patients and controls by determining plasma levels of 8-OHdG, as a conventional biomarker. We showed that 8-OHdG concentration was higher in ccRCC patients compared to controls (1.00 ng/ml vs. 0.70 ng/ml, respectively). In attempt to discern functional role of *GSTO1* and *GSTO2* polymorphisms, we stratified 8-OHdG levels of ccRCC patients by *GSTO1* (rs4925) and *GSTO2* (rs156697, rs2297235) genotypes. The plasma concentration of 8-OHdG, a biomarker of oxidative DNA damage, was significantly higher in patients with *GSTO2**G/G variant genotype (rs2297235) (1.41ng/ml) than in carriers of at least one *GSTO2**A referent allele (0.99 ng/ml) (p=0.042). Regarding GSTO1 polymorphism, 8-OHdG levels were higher in carriers of variant *GSTO1**A/A genotype, however, the statistical significance was not reached. In contrast, no relation was found between plasma 8-OHdG levels and *GSTO2* (rs156697) genotypes (Table 8).

Table 8. The concentration of 8-OHdG in plasma of ccRCC patients stratified according to *GSTO1* and *GSTO2* genotypes

Genotype	8-OHdG in ccRCC patients (ng/ml)[a]	p
***GSTO1* rs4925**		
*C/C+*C/A	0.99 (0.39-1.80)	
*A/A	1.21 (0.92-1.61)	0.154
***GSTO2* rs156697**		
*A/A+*A/G	1.00 (0.39-1.80)	
*G/G	1.09 (0.62-1.61)	0.448
***GSTO2* rs2297235**		
*A/A+*A/G	0.99 (0.39-1.62)	
*G/G	1.41 (0.92-1.80)	0.042

[a]Median (min-max); $p<0.05$ was considered to be statistically significant

4.2 Relevance of glutathione transferase omega class gene polymorphisms in prognosis of ccRCC patients

Prognostic significance of *GSTO1* (rs4925) and *GSTO2* (rs156697, rs2297235) polymorphisms was evaluated in 239 patients with ccRCC.

4.2.1 The relevance of *GSTO1* and *GSTO2* polymorphisms in overall survival of ccRCC patients

The effect of GSTO genotypes on overall survival was investigated in patients with ccRCC, the most common and apparently the most aggressive RCC subtype. Among 228 ccRCC patients with successfully obtained follow-up information there were 79 (35%) deaths during the follow-up period. The median follow-up was 67 months ranging from 1 to 153 months.

In *Kaplan-Meier* survival analysis *GSTO1* (rs4925) and *GSTO2* (rs156697 and rs2297235) polymorphisms did not show effect on overall survival among ccRCC patients (Figures 9-11).

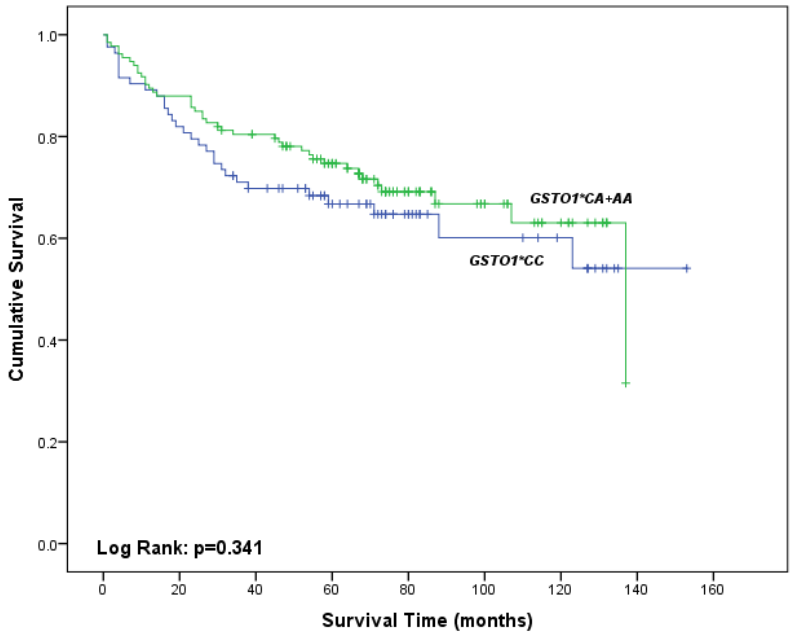

Figure 9. Overall survival of ccRCC patients stratified by *GSTO1* rs4925 polymorphism

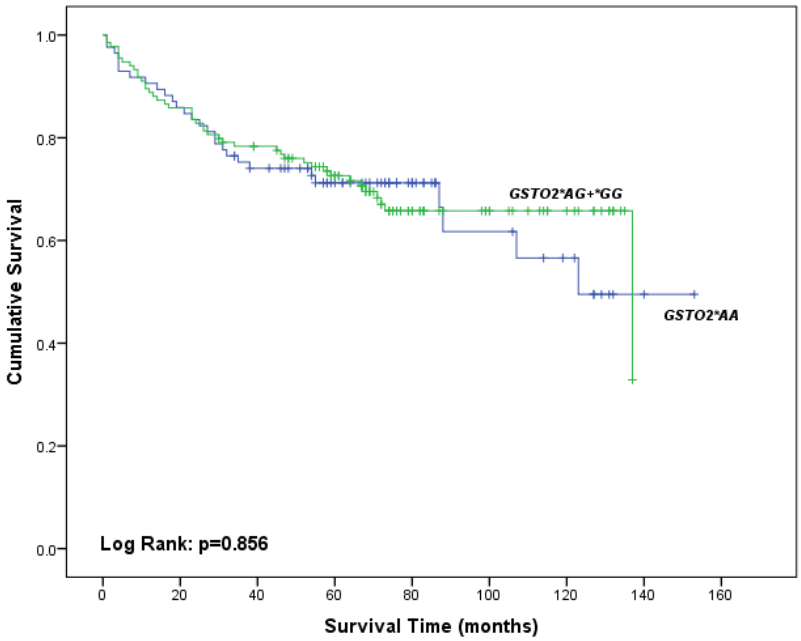

Figure 10. Overall survival of ccRCC patients stratified by *GSTO2* rs156697 polymorphism

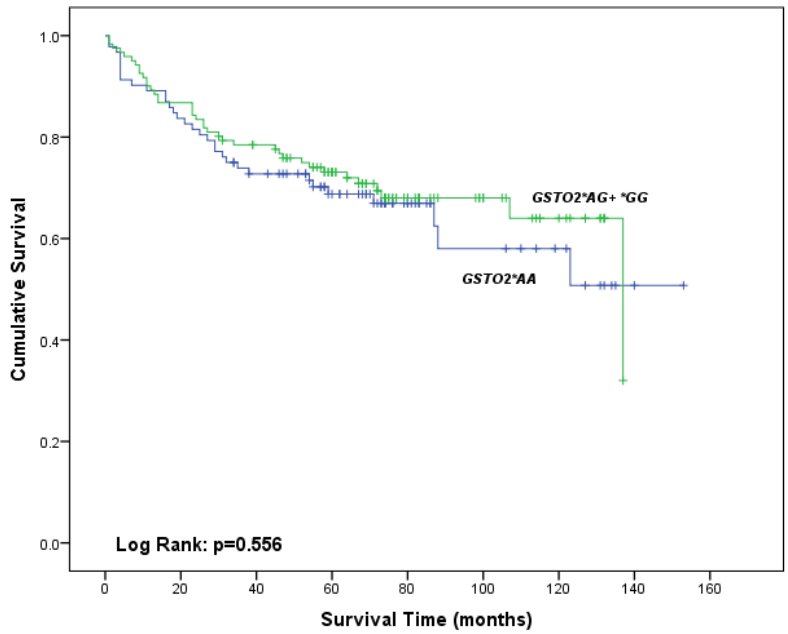

Figure 11. Overall survival of ccRCC patients stratified by *GSTO2* rs2297235 polymorphism

Table 9 demonstrates the associations between GSTO genotypes and overall mortality, adjusted by Fuhrman nuclear grade and pT stage, as recognized prognostic factors of RCC. The multivariate *Cox* regression analysis did not demonstrate statistically significant association between any of the GSTO genotypes analyzed and overall mortality among ccRCC patients.

Table 9. Predicting effect of GSTO polymorphisms on overall mortality in ccRCC patients

		Events, n (%)	HR (95% CI)[b]	p
***GSTO1* rs4925**				
FNR[a]	G1/G2/G3/G4	2 (7)/32 (29)/29 (58)/5 (63)	1.57 (1.08-2.27)	0.017
pT stage	pT1/pT2/pT3/pT4	14 (14)/8 (33)/ 51 (57)/3 (60)	2.01 (1.46-2.76)	<0.001
GSTO1 rs4925				
*CC		32 (38)	1.53 (0.91-2.58)	0.107
*CA+*AA		47 (34)	1.00	
***GSTO2* rs156697**				
FNR	G1/G2/G3/G4	2 (7)/32 (29)/29 (58)/5 (63)	1.58 (1.09-2.27)	0.015
pT stage	pT1/pT2/pT3/pT4	14 (14)/8 (33)/51 (57)/3 (60)	1.97 (1.43-2.70)	<0.001
GSTO2 rs156697				
*AA		31 (35)	1.11 (0.66-1.88)	0.689
*AG+*GG		48 (34)	1.00	
***GSTO2* rs2297235**				
FNR	G1/G2/G3/G4	2 (7)/32 (29)/29 (58)/5 (63)	1.57 (1.08-2.27)	0.016
pT stage	pT1/pT2/pT3/pT4	14 (14)/8 (33)/51 (57)/3 (60)	1.96 (1.43-2.69)	<0.001
GSTO2 rs2297235				
*AA		34 (36)	1.24 (0.74-2.07)	0.425
*AG+*GG		44 (34)	1.00	

[a]Fuhrman nuclear grade; [b]HR, odds ratio adjusted to Fuhrman nuclear grade and pT stage; CI, confidence interval; $p<0.05$ was considered to be statistically significant

4.2.2 The relevance of *GSTO1* and *GSTO2* polyporphisms in overall survival of male ccRCC patients

Considering the male predominance in RCC, we further focused on evaluation of the potential effect of different GSTO genotypes on overall survival in male ccRCC patients. Among 154 men with successfully obtained follow-up information there were 61 (40%) deaths during the follow-up period.

Kaplan-Meier survival analysis indicated shorter overall survival (log-rank: p=0.049) in male carriers of *GSTO1**C/C wild type genotype compared to the male carriers of at least one variant allele (Figure 12). However, *GSTO2* (rs156697 and rs2297235) polymorphisms did not show effect on overall survival among male ccRCC patients (Figures 13 and 14).

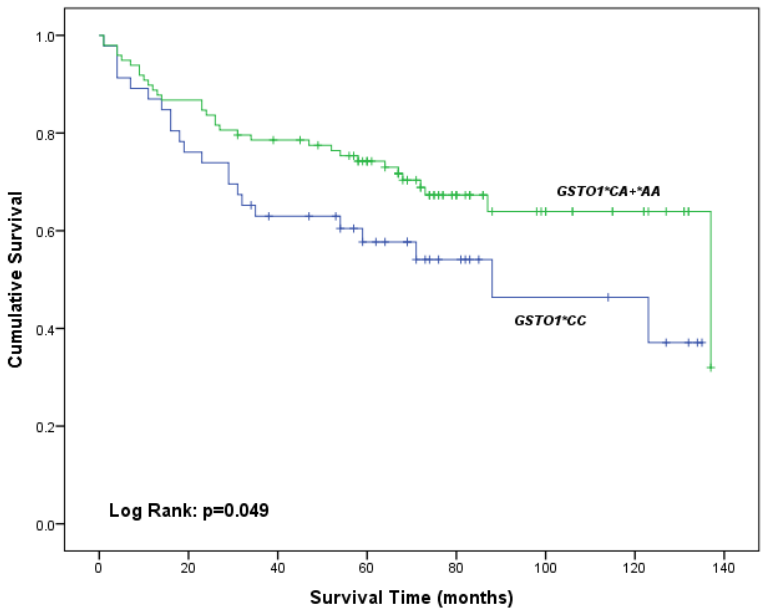

Figure 12. Overall survival of male ccRCC patients stratified by *GSTO1* rs4925 polymorphism

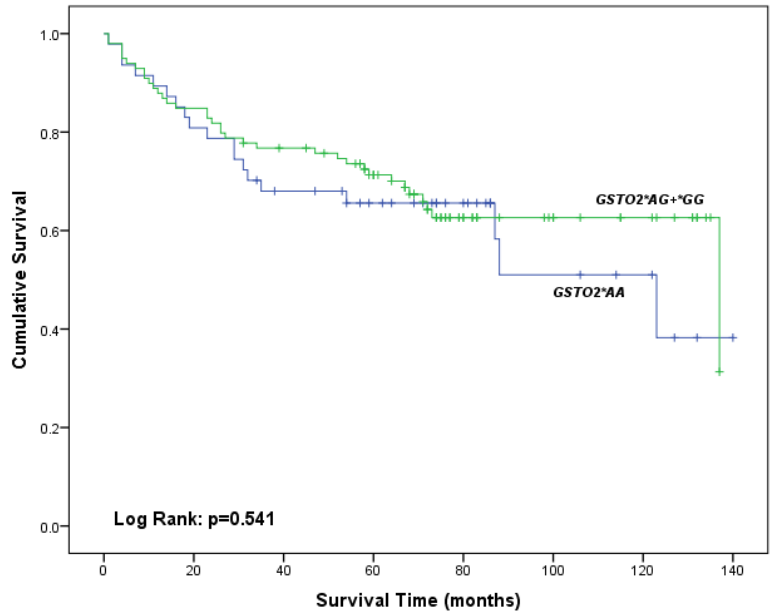

Figure 13. Overall survival of male ccRCC patients stratified by *GSTO2* rs156697 polymorphism

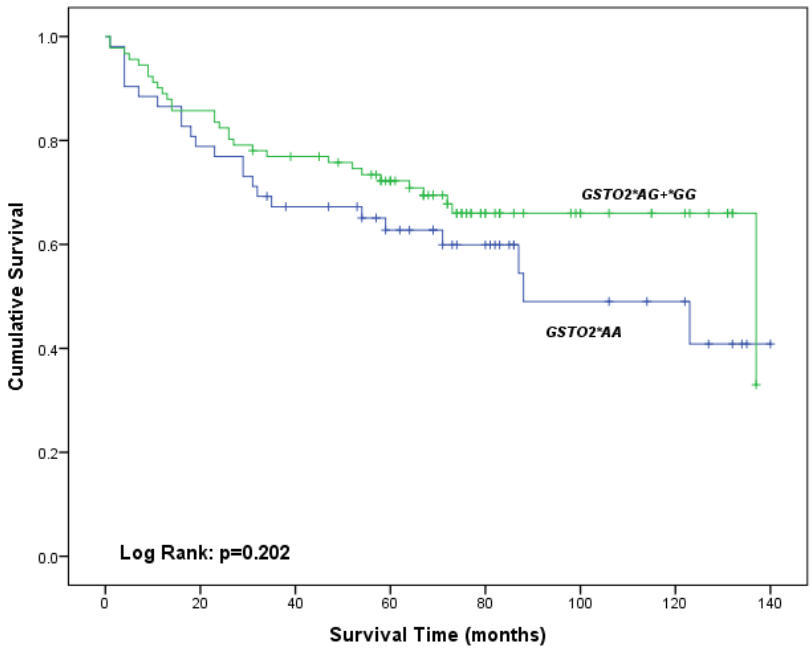

Figure 14. Overall survival of male ccRCC patients stratified by *GSTO2* rs2297235 polymorphism

Table 10 demonstrates the associations between different GSTO genotypes and overall mortality, adjusted by Fuhrman nuclear grade and pT stage, among male ccRCC patients. The multivariate *Cox* regression analysis confirmed *GSTO1**CC genotype as an independent predictor of higher risk for overall mortality in patients with male ccRCC. Namely, male carriers of *GSTO1**CC genotype had significantly increased hazard ratio compared to the carriers of *GSTO1**A allele (HR=1.89, 95%CI:1.04-3.42, p=0.037). Regarding *GSTO2* (rs156697 and rs2297235) genotypes, the results did not reach statistical significance(p>0.05, Table 10).

Table 10. Predicting effect of GSTO polymorphisms on overall mortality in male ccRCC patients

		Events, n (%)	HR (95% CI)[b]	p
***GSTO1* rs4925**				
FNR[a]	G1/G2/G3/G4	2 (12)/23 (30)/26 (72)/2 (40)	1.58 (1.03-2.43)	0.037
pT stage	pT1/pT2/pT3/pT4	10(16)/5 (36)/42 (61)/2 (50)	1.83 (1.28-2.62)	0.001
GSTO1 rs4925				
*CC		23 (49)	1.89 (1.04-3.42)	0.037
*CA+*AA		38 (36)	1.00	
***GSTO2* rs156697**				
FNR	G1/G2/G3/G4	2 (12)/23 (30)/26 (72)/2 (40)	1.56 (1.02-2.38)	0.040
pT stage	pT1/pT2/pT3/pT4	10(16)/5 (36)/42 (61)/2 (50)	1.83 (1.29-2.60)	0.001
GSTO2 rs156697				
*AA		21 (43)	1.32 (0.71-2.43)	0.380
*AG+*GG		40 (38)	1.00	
***GSTO2*rs2297235**				
FNR	G1/G2/G3/G4	2 (12)/23 (30)/26 (72)/2 (40)	1.59 (1.04-2.46)	0.034
pT stage	pT1/pT2/pT3/pT4	10(16)/5 (36)/42 (61)/2 (50)	1.81 (1.27-2.59)	0.001
GSTO2 rs2297235				
*AA		24 (45)	1.60 (0.88-2.92)	0.127
*AG+*GG		36 (37)	1.00	

[a]Fuhrman nuclear grade; [b]HR, odds ratio adjusted to Fuhrman nuclear grade and pT stage; CI, confidence interval; p<0.05 was considered to be statistically significant

4.3 Protein expression of glutathione transferase omega class and downstream effectors of PI3K/Akt and Raf/MEK/ERK signaling pathway in ccRCC tumor and corresponding non-tumor tissue

Tumor and corresponding non-tumor tissue specimens were taken during total nephrectomy from 30 patients with ccRCC. All tumor samples were categorized by their pT stage to early-stage (pT1 and pT2) and late-stage (pT3 and pT4) ccRCC. Cytocolic fraction was used for determination of protein expression profile of ccRCC, as well as immunoprecipitation analysis.

4.3.1 GSTO1 protein expression

Densitometry analysis of data provided by Western blot showed 1.5-fold higher expression of GSTO1 protein in tumor ccRCC samples compared to their respective non-tumor tissue samples (p=0.002, Figure 15). Representative blots demonstrating increased protein expression of GSTO1 in tumor compared to corresponding non-tumor specimens are presented in the Figure 15. Expression of GSTO1 was increased in 18, decreased in 5, and unchanged in 2 tumor samples compared to corresponding non-tumor tissue.

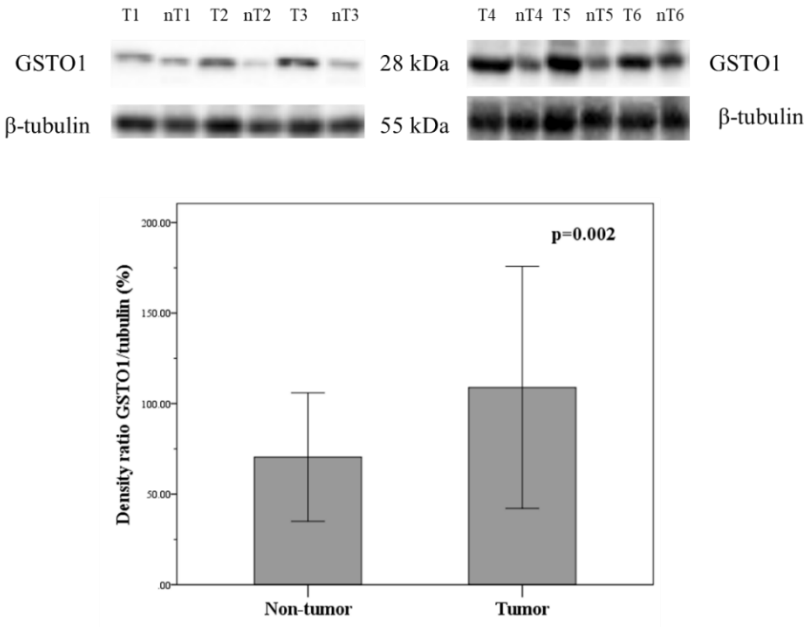

Figure 15. Expression of GSTO1 protein (28 kDa) in ccRCC tumor (T) and corresponding non-tumor (nT) tissue samples

Furthermore, in tumor samples stratified according to pT stage, statistically significant decrease of GSTO1 protein expression in the late-stage compared to early-stage ccRCC was found (p=0.044, Figure 16).

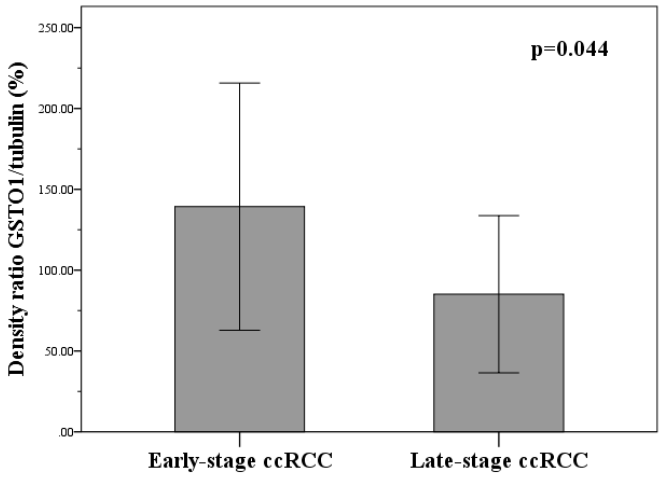

Figure 16. Expression of GSTO1 protein (28 kDa) in tumor ccRCC tissue samples according to pT stage of ccRCC; early-stage ccRCC- pT1 and pT2; late-stage ccRCC- pT3 and pT4

Additionally, no statistical significance in GSTO1 protein expression when stratified according to Fuhrman nuclear grade of ccRCC was found (Figure 17).

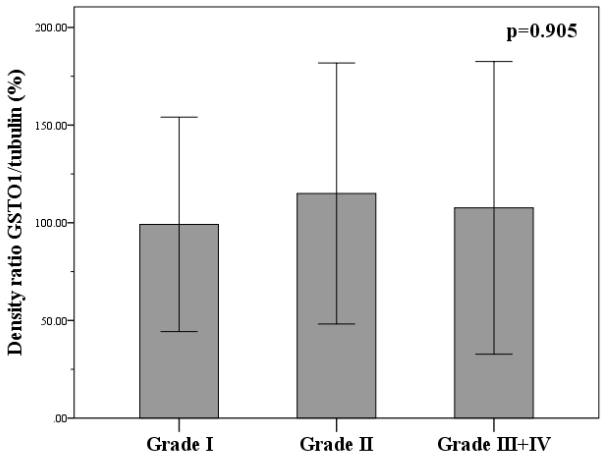

Figure 17. Expression of GSTO1 protein (28 kDa) in tumor ccRCC tissue samples according to Fuhrman nuclear grade of ccRCC

4.3.2 GSTO2 protein expression

Densitometry analysis of data provided by Western blot showed 2.2-fold higher expression of GSTO2 protein in tumor ccRCC samples compared to their respective non-tumor tissue samples (p=0.007, Figure 18). Representative blots demonstrating increased protein expression of GSTO2 in tumor compared to corresponding non-tumor specimens are presented in the Figure 18. Expression of GSTO2 was increased in 18, decreased in 5, and unchanged in 3 tumor samples compared to corresponding non-tumor tissue.

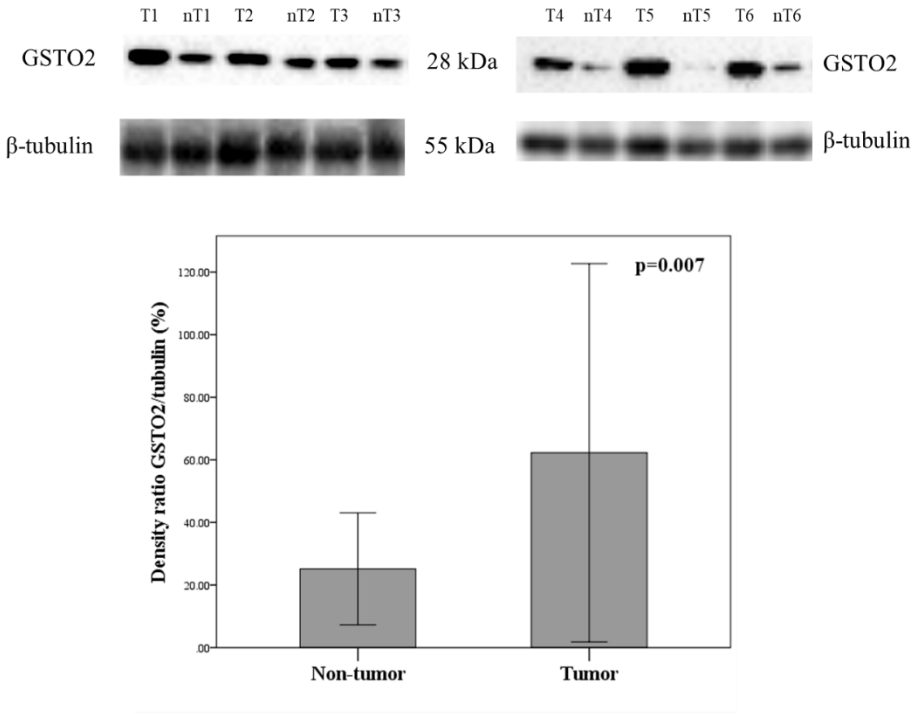

Figure 18. Expression of GSTO2 protein (28 kDa) in ccRCC tumor (T) and corresponding non-tumor (nT) tissue samples

Furthermore, in tumor samples stratified according to pT stage, decrease of GSTO2 protein expression in the late-stage compared to early-stage ccRCC was observed, but this change did not reach statistical significance (p=0.274, Figure 19).

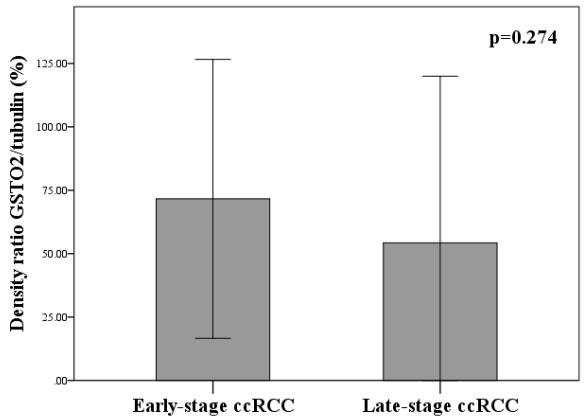

Figure 19. Expression of GSTO2 protein (28 kDa) in tumor ccRCC tissue samples according to pT stage of ccRCC; early-stage ccRCC- pT1 and pT2; late-stage ccRCC- pT3 and pT4

Additionally, no statistical significance in GSTO2 protein expression when stratified according to Fuhrman nuclear grade of ccRCC was found (Figure 20).

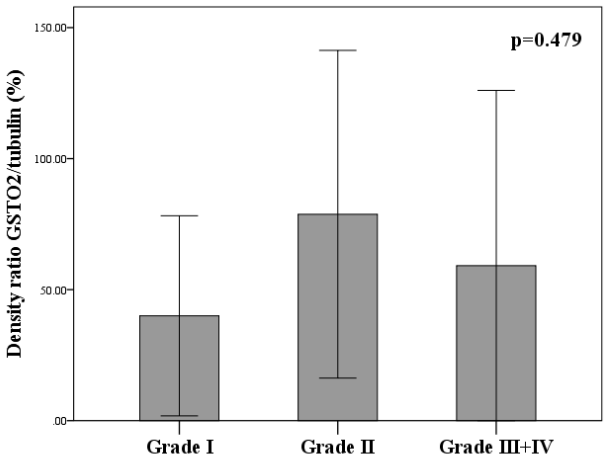

Figure 20. Expression of GSTO2 protein (28 kDa) in tumor ccRCC tissue samples according to Fuhrman nuclear grade of ccRCC

4.3.3 Phosphorylation status of downstream effectors of PI3K/Akt/mTOR and Raf/MEK/ERK signaling pathways in ccRCC

Considering important role of two pro-survival pathways, PI3K/Akt/mTOR and Raf/MEK/ERK in ccRCC, we assessed phosphorylation status of their downstream effectors. PI3K downstream effectors, specifically targeted by the antibody cocktail used, are Akt1 phospho S473 and RPS6 phospho S235/236, whereas the downstream effectors of the MEK pathway are ERK1/2 phospho Y204/197 and p90RSK phospho S380. These two pathways are known for promoting cell growth, regulating apoptosis, chemotherapeutic drug resistance and cellular senescence. Both pathways affect protein translation by complex interactions regulating mTORC1/2 complexes; they regulate each other as well as other pathways such as Wnt/β-catenin, Jak/STAT, NF-κB and TGFβ.

This study showed increased expression of RSK1p90 phospho S380, Akt1 phospho S473, ERK1/2 phospho Y204/197 and RPS6 phospho S235/236 in tumor ccRCC tissue compared to corresponding non-tumor tissue (Figure 21).

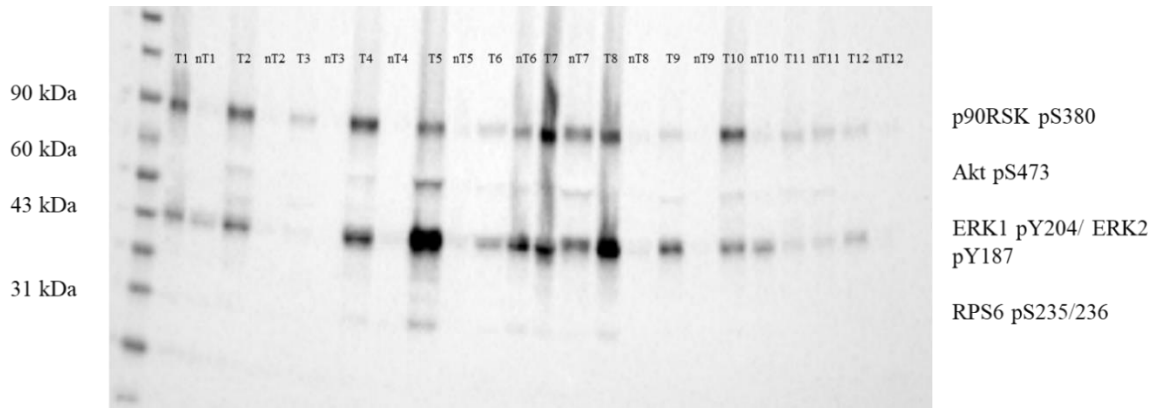

Figure 21. Phosphorylation status of downstream effectors of PI3K/Akt/mTOR and Raf/MEK/ERK signaling pathways in ccRCC tumor (T) and corresponding non-tumor (nT) tissue samples; RSK1p90- 90 kDa ribosomal protein S6 kinase 1; Akt-protein kinase B; ERK- extracellular signal-regulated kinase; RPS6-ribosomal protein S6

4.3.4 Immunoprecipitation of GSTO1 and associated proteins in tumor ccRCC tissue

Furthermore, we examined possible association of GSTO1 with downstream effectors of PI3K/Akt/mTOR and Raf/MEK/ERK signaling pathways shown to be upregulated in ccRCC tissue. After protein immunoprecipitation of ccRCC tumor tissue samples by anti-GSTO1 antibody Western blot analysis showed an association of GSTO1 with RSK1p90 phospho S380, RPS6 phospho S235/236 and Akt1 phospho S473 (Figure 22). Interestingly, ERK1/2 phospho Y204/197 did not co-immunoprecipitated with GSTO1. Furthermore, beside molecules targeted by the antibody cocktail, we demonstrated association of GSTO1 with other phosphorylated form of Akt (Akt1 pT308) and total Akt (panAkt) (Figure 22). Additionally, association of β-actin with GSTO1 was shown (Figure 22). This association was expected knowing that β-actin is the target for GSTO1 deglutathionylase activity.

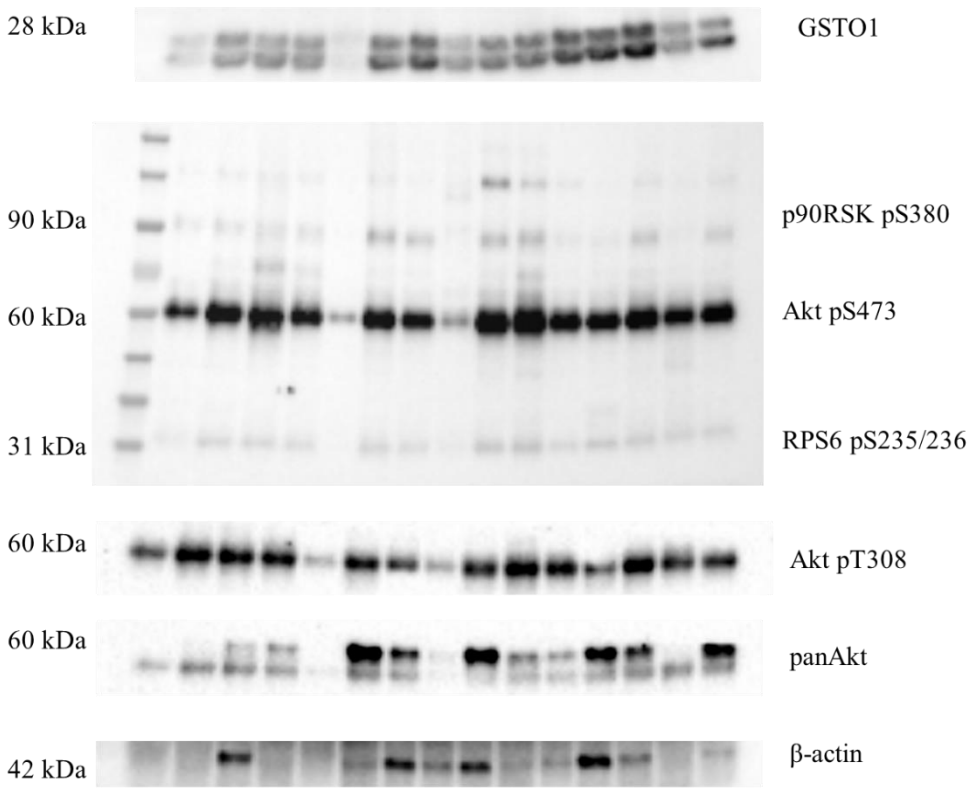

Figure 22. Immunoprecipitation of GSTO1 and associated proteins in tumor ccRCC tissue samples; RSK1p90- 90 kDa ribosomal protein S6 kinase 1; Akt-protein kinase B; ERK- extracellular signal-regulated kinase; RPS6-ribosomal protein S6

4.3.5 Correlation between GSTO1 expression and IL-1β/pro-IL-1β ratio in tumor ccRCC tissue

Considering the role of IL-1β in ccRCC tumor progression and the role of GSTO1 in posttranslational processing of IL1-β, we assessed pro-IL-1β and IL-1β levels in tumor ccRCC tissue samples. We analyzed correlation between GSTO1 protein expression and IL-1β/pro-IL-1β ratio in tumor ccRCC tissue samples. Weak positive correlation was found between GSTO1 and IL-1β/ pro-IL-1β ratio (r=0.260, p=0.350) (Figure 23).

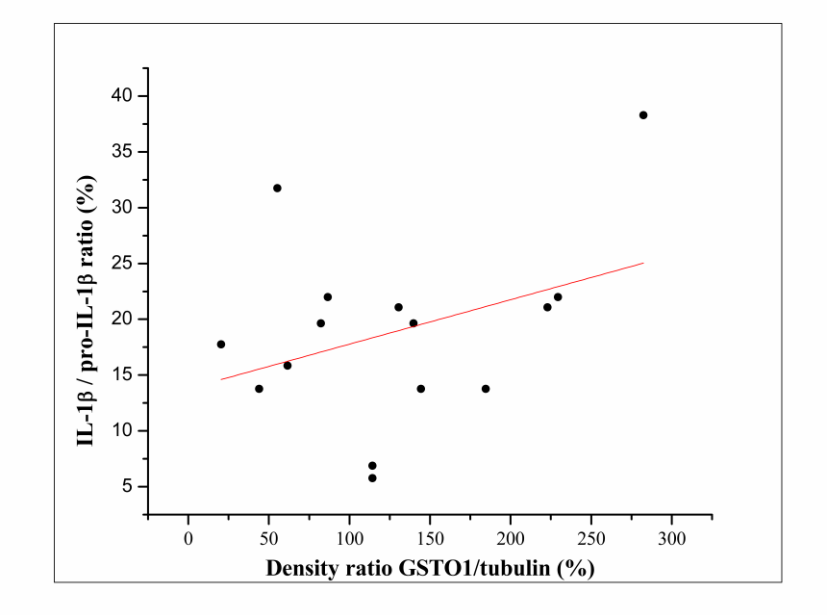

Figure 23. Correlation between GSTO1 and IL-1β/ pro-IL-1β ratio in tumor ccRCC tissue samples

5 DISCUSSION

Renal cell carcinoma represents a group of histologically similar neoplasms characterized by significant intra- and inter-tumor genetic heterogeneity. Among different subtypes of RCC, the highest incidence and the most aggressive phenotype was shown for the clear cell RCC (ccRCC). Multistage processes of RCC development and progression are characterized by altered cellular metabolism and numerous genetic, epigenetic and proteomic changes. In addition to recognized role of VHL inactivation and impaired redox homeostasis in the development and progression of RCC, changes in GST expression profile might be important contributing factor regulating signaling pathways involved in cell proliferation and survival. Indeed, there are evidence on implication of GSTs in RCC risk, as well as progression and prognosis (Pljesa-Ercegovac et al., 2019).

GSTs are multifunctional enzymes exhibiting various catalytic functions, still traditionally recognized as phase II cellular detoxification system enzymes (Hayes et al., 2005; Pljesa-Ercegovac et al., 2018). In addition to their well-established catalytic roles, GSTs have also become known as regulators of cell proliferation and survival signaling pathways (Board and Menon, 2013). In comparison to other GST classes, omega class (GSTO) possesses intriguing range of both catalytic and non-catalytic roles. It seems that their thioltransferase, dehydroascorbate reductase (DHAR) and deglutahionylase activities contribute to regulation of redox homeostasis (Board and Menon, 2016). In this context, GSTO2-2 exhibits powerful DHAR activity (Zhou et al., 2012), while GSTO1-1 has been found to play a major role in the glutathionylation cycle that is emerging as significant mechanism regulating protein function by catalyzing both the glutathionylation and deglutathionylation (Menon and Board, 2013). Furthermore, GSTO1-1 has several regulatory roles, including modulation of posttranslational processing of pro-IL1β to its active form, as well as modulation of ryanodine receptors (Dulhunty et al., 2001; Laliberte et al., 2003). In addition, anti-apoptotic and pro-survival role of GSTO1-1 emerged as important aspect of chemoresistance in several cancer cell lines (Piaggi et al., 2010; Yan et al., 2007).

Significant gene heterogeneity was observed in the omega class GSTs due to the presence of single nucleotide polymorphisms and deletions. Mukherjee et al. described 31 polymorphisms in *GSTO1* and 66 polymorphisms in the *GSTO2* gene (Mukherjee et

al., 2006). Until now, significant association between *GSTO1* rs4925 polymorphism and risk of acute childhood lymphoblastic leukemia (Pongstaporn et al., 2009), hepatocellular carcinoma, cholangiocarcinoma (Marahatta et al., 2006), and non-small cell lung cancer (Ada et al., 2013) has been reported. In contrast to recognized association of *GSTO1* rs4925 variant allele with susceptibility to various cancers, *GSTO1**A/A variant genotype might be related to lower aggressiveness of head and neck squamous cell carcinoma (Sanguansin et al., 2012). Regarding *GSTO2**A424G polymorphism (rs156697), it has been shown that *GSTO2**G variant allele increased the risk of ovarian cancer, however, this association was not statistically significant (Pongstaporn et al., 2006). Several studies investigated potential role of *GSTO* polymorphisms in susceptibility to bladder cancer (Djukic et al., 2015; Lesseur et al., 2012; Wang et al., 2009). Wang et al. found that carriers of *GSTO2**G/G genotype (rs2297235) exhibit significantly higher risk for bladder cancer development (Wang et al., 2009). Additionally, they showed that carriers of haplotype comprised of *GSTO1**C wild type (rs4925), *GSTO2**G variant (rs156697) and *GSTO2**G variant (rs2297235) alleles had increased bladder cancer risk compared to haplotype consisting of all three wild type alleles (Wang et al., 2009). Two studies showed association of *GSTO2**G/G genotype (rs156697) with higher bladder cancer risk (Djukic et al., 2015; Lesseur et al., 2012). Results by Djukic et al. also confirmed association of *GSTO1**C (rs4925)/ *GSTO2**G (rs156697) haplotype with higher bladder cancer risk (Djukic et al., 2015). Interestingly, there are reports on association of *GSTO2**A/A referent type genotype (rs156697) with increased risk for colorectal cancer in individuals with positive family history for cancer (Masoudi et al., 2011) and protective role of *GSTO2**G/G variant genotype (rs156697) regarding the risk of gastric cancer (Masoudi et al., 2009). Data on GSTO polymorphisms in relation to breast cancer risk are inconsistent (Andonova et al., 2010; Marahatta et al., 2006; Olsen et al., 2008). Lately, meta-analysis performed to investigate the association strength of GSTO polymorphisms with cancer risk concluded that *GSTO2* rs156697 polymorphism might be associated with higher risk of breast cancer (Xu et al., 2014).

This study represents the first comprehensive research on the relevance of polymorphisms and expression profiles of novel GST omega class in ccRCC subtype. We investigated the potential role of *GSTO1* (rs4925) and *GSTO2* (rs156697 and

rs2297235) polymorphisms as determinants of both risk and postoperative prognosis in ccRCC patients. Furthermore, in non-tumor and tumor ccRCC tissue, we assessed GSTO1-1 and GSTO2-2 expression, as well as phosphorylation status of PI3K/Akt/mTOR and Raf/MEK/ERK signaling pathways. Possible association of GSTO1-1 with signaling molecules known to be regulated by glutathionylation was also studied.

The effect of the most studied SNPs, *GSTO1* rs4925 and *GSTO2* rs156697, and less investigated *GSTO2* rs2297235 independently and in conjuction with recognized risk factors (smoking, obesity and hypertension) on the ccRCC was evaluated. The results have shown that subjects with combined variant *GSTO1* (rs4925) and *GSTO2* (rs156697 and rs2297235) genotypes exhibit 2.6-fold higher risk of developing ccRCC in comparison with the carriers of combined wild-type genotypes. Although *GSTO2* rs156697 polymorphism increases the risk of ccRCC, the statistical significance was reached only when analyzed in combination with *GSTO1* rs4925 polymorphism or smoking. Considering demonstrated significant linkage disequilibrium of GSTO genes, we also evaluated potential impact of *GSTO1* and *GSTO2* haplotypes on ccRCC risk. We showed that the carriers of H2 haplotype, comprised of *GSTO1**A (rs4925), *GSTO2**G (rs156697) and *GSTO2**G (rs2297235) variant alleles, exhibited 1.5-fold higher ccRCC risk in comparison with carriers of H1 haplotype, comprised of all three referent alleles. Considering GSTO1 and GSTO2 antioxidant and regulatory activities, these results imply the relevance of the GSTO SNPs in inter-individual susceptibility to oxidative stress. Namely, the presence of H2 haplotype, exhibiting low deglutathionylase and low DHAR activity, might underlie the altered redox homeostasis and influence propensity for ccRCC development.

It has been shown that variant *GSTO1**A allele has lower deglutathionylase activity and higher activity in the forward glutathionylation reaction in contrast to *GSTO1**C wild-type allele (Menon and Board, 2013; Tanaka-Kagawa et al., 2003). The selective glutathionylation or deglutathionylation of specific protein thiols contributes to numerous cellular processes involved in tumor growth, such as cell cycle regulation, cytoskeleton remodeling, epigenetic DNA modifications and apoptosis (Menon and Board, 2013). Since GSTO1-1 exhibits specificity for particular proteins or particular glutathionylated cysteine residues (Board and Menon, 2016), the presence of *GSTO1*

allelic variants with altered activity might provide a conceivable mechanism to elucidate possible link between *GSTO1* rs4925 polymorphism and different cancers (Xu et al., 2014). In addition to *GSTO1*, it appears that the most commonly investigated *GSTO2* polymorphism (rs156697) might also affect primarily its antioxidant DHAR activity (Piacentini et al., 2013; Whitbread et al., 2005), which is important for regulating the cellular ascorbic acid redox state. It seems that this reaction might be meaningful in solid tumors. Namely, some colorectal tumor cell lines have shown higher uptake of dehydroascorbate by the GLUT1 transporters (Yun et al., 2015). Since higher expression of GLUT1 transporters is common feature of different solid tumors, including ccRCC, this phenomenon of increased dehydroascorbate uptake requires further clarification. Moreover, ascorbic acid and Fe(II), 2-oxoglutarate act as cofactors of oxygen–dependent protein hydroxylases, the main regulators of HIF activity. Namely, these enzymes mark HIFα for ubiquitinylation and consequential proteasomal degradation (Mehdi and Riazalhosseini, 2017). In that context, it might be hypothesized that ascorbic acid-dependent inhibition of the HIF signaling might provide additional approach for managing tumor progression and inflammation (Li and Schellhorn, 2007). As a possible consequence of *GSTO2* polymorphism, diminished regeneration of ascorbic acid, might also influence HIFα hydroxylation and promotes its accumulation. As a result, downstream overexpression of HIF-dependent genes involved in metabolic shift towards glycolysis, angiogenesis, proliferation, cell survival, migration and invasion, could contribute to ccRCC progression (Mehdi and Riazalhosseini, 2017). Still, it can be suggested that low DHAR activity in ccRCC patients carriers of both variant *GSTO2* alleles decreases the ratio between reduced and oxidized form of ascorbic acid and contributes to impaired redox homeostasis as hallmark of malignant ccRCC phenotype. Further, in terms of oxidative phenotype, we investigated oxidative DNA modifications in ccRCC patients stratified by *GSTO1* and *GSTO2* genotypes, by determining the plasma levels of 8-OHdG as the most suitable biomarker (Valavanidis et al., 2009a). We found statistically significant increase in 8-OHdG plasma concentration in ccRRC patients, carriers of *GSTO2**G/G variant genotype (rs2297235) compared to carriers of referent allele. This is in line with our previous data on higher urinary 8-OHdG levels in bladder cancer patients, carriers of *GSTM1-null* and *GSTA1-*

variant genotypes, known to contribute to diminished antioxidant capacity (Savic-Radojevic et al., 2013).

Regarding the gene-environment interactions and ccRCC risk, smokers with variant *GSTO2* rs156697 genotype were at higher risk in comparison with non-smokers carriers of at least one referent allele. It seems that smoking, as an important source of ROS (Valavanidis et al., 2009b), contributes to genotype-associated ccRCC risk in carriers of *GSTO2*-variant genotype. Concerning other risk factors associated with RCC, such as hypertension and obesity, no modifying effect of GSTO genotypes was found in this study. Hypertension alone was significantly associated with the development of ccRCC, once again confirming hypertension as independent risk factor for RCC (Hsieh et al., 2017b). Considering that strong association between weight gain in early and mid-adulthood (18-35 years of age) and RCC was found (Hsieh et al., 2017b), more detailed acquisition of data regarding change of BMI during patients' life would be necessary to investigate effect of excess body weight on ccRCC risk.

In addition to modifying effect of GSTO polymorphism in terms of ccRCC risk, the prognostic significance of these polymorphisms was also demonstrated in our study for the first time. Our results indicated shorter survival in male carriers of *GSTO1**C/C referent type genotype compared to the carriers of at least one variant allele. Moreover, *GSTO1**C/C referent type genotype independently predicted higher risk of overall mortality among male ccRCC patients when the association between different GSTO genotypes and overall mortality, adjusted by recognized prognostic factors, was analyzed. Interestingly, no statistically significant results were obtained for investigated polymorphisms in terms of postoperative prognosis and the risk of overall mortality when the whole group, regardless of gender, was analyzed. Considering that men are more affected by RCC than women and slightly different modifying effect of risk factors in two populations (Hsieh et al., 2017b), it is acceptable that some mechanisms underlying disease progression might be different. Possible rationale of prognostic significance of *GSTO1* polymorphism in ccRCC patients might be the role of GSTO1 in modulation of posttranslational processing of IL-1β (Laliberte et al., 2003). It has been known that high serum levels of IL-1β are associated with advanced disease in RCC patients (Yoshida et al., 2002). Moreover, tumor-associated macrophages found in aggressive RCC tumors express high levels of IL-1β (Ikemoto et al., 2003). Petrella and

Vincenti elucidated that the role of IL-1β in tumor progression might be the stimulation of tumor cell invasion of RCC cells in a process that was dependent on the activity of MMPs. IL-1β induced the expression of MMPs by the activation of the transcription factor CCAAT enhancer binding protein β (CEBP β) (Petrella and Vincenti, 2012). Considering that the role of GSTO1-1 in post-translational processing of IL-1β could be mediated by its glutathionylase/deglutathionylase activity that is dependent on *GSTO1* allelic variant (Menon and Board, 2013), it seems plausible that *GSTO1* polymorphism could affect activation of IL-1β and tumor progression.

Further investigations have been focused on the potential molecular mechanisms underlying the GSTO involvement in ccRCC progression. The expression profile of both GSTO isoenzymes, together with phosphorylation status of PI3K/Akt and MAPK/ERK signaling pathways, known to be constitutively active, in ccRCC tumor and corresponding non-tumor tissue were determined. In addition to findings on significant GSTO1-1 upregulation in ccRCC, our results also demonstrated the change in expression levels between early-stage and late-stage ccRCC. The GSTO1-1 increased expression has been reported in different cancers, including bladder (Djukic et al., 2017), pancreatic (Chen et al., 2009), ovarian cancer (Urzúa et al., 2006; Yan et al., 2007) and esophageal adenocarcinoma (Li et al., 2014). In addition, nuclear localization of GSTO1-1 in Barrett's esophagus (Piaggi et al., 2009), however, also in colorectal carcinoma (Lombardi et al., 2015) suggests its potential role in the protection of specific nuclear components against the oxidative stress, in that way, contributing to malignant transformation.

Interestingly, we found significant difference between early-stage and late-stage ccRCC regarding GSTO1 expression levels. Namely, GSTO1 expression was significantly higher in early-stage ccRCC tumor tissue in comparison to late-stage ccRCC. Throughout ccRCC progression, complex changes of redox homeostasis accomplished with metabolic shift contribute to survival of tumor cells (Lusini et al., 2001). By modifying primarily their metabolic phenotype, cancer cells try to maintain steady-state of high ROS levels within a narrow range, which allows them to increase growth and invasion while limit their apoptotic propensity (Rodic and Vincent, 2018). In this manner are also data on the shift in GSH/GSSG ratio between early- and late-stage RCC, which is associated with decrease of numerous enzymes involved in GSH

metabolism (glutathione peroxidase, glutathione transferase, γ-glutamyl transpeptidase, glutathione reductase) only in early-stage RCC (Lusini et al., 2001; Pljesa-Ercegovac et al., 2008). It might be speculated that GSTO1 can affect evolution of ccRCC by at least two mechanisms. Namely, higher deglutathionylase activity potentiates oxidative stress and increases susceptibility to oxidative damage by exposing molecules to oxidative modifications in early-stage RCC. Besides, more importantly, deglutathionylation seem to regulate and modify biological activity of the affected proteins, which will be further discussed in more detail. Based on study of Menon and Board (Menon and Board, 2013) revealing the GSTO1-1 role in glutathionylation cycle, we assumed that increased GSTO1 expression in ccRCC might significantly affect a regulation of redox-sensitive signaling pathways by its deglutathionylase activity. Until now, the regulatory role of glutathionylation in redox signaling was investigated concerning mainly glutaredoxins as the major intracellular deglutathionylating enzyme. Even more, the relevance of glutathionylation status in signaling regulation was confirmed by manipulation of glutaredoxin levels, which significantly affected signaling events (Menon and Board, 2013). Regulation through S-glutathionylation has been attributed to a large number of proteins involved in signaling (kinases and phosphatases), protein folding and stability, redox homeostasis, calcium homeostasis; cytoskeletal; transcription factors; heat shock proteins; energy metabolism and glycolysis (Zhang et al., 2018). Until know, the potential target subjected for GSTO1-1-mediated regulation by deglutathyonylation are β-actin, heat shock protein 70, heat shock protein 7c and prolactin-inducible protein (Menon and Board, 2013).

Moreover, our results on correlation between IL-1β/pro- IL-1β ratio, as measure of activation IL-1β, and level of GSTO1 protein expression in ccRCC tumor tissue is expected, considering recognized role of GSTO1-1 in modulation of posttranslational processing of IL-1β (Laliberte et al., 2003). For the assessment of stronger correlation larger study would be warranted.

Similar pattern of protein expression was shown for the GSTO2-2. Namely, significantly higher protein expression of GSTO2 in tumor ccRCC tissue compared to non-tumor tissue was found. Furthermore, decrease of GSTO2 protein expression in the late-stage compared to early-stage ccRCC was observed, however, this change did not

reach statistical significance. The role of upregulated GSTO2-2 in ccRCC remains unclear.

The PI3K/Akt signaling pathway is considered to have pivotal role in the regulation of proliferation, differentiation and survival of ccRCC cancer cells (Wu et al., 2019). This signaling pathway is highly activated in ccRCC, as demonstrated in both cancer cell lines and ccRCC tumor tissue. Numerous downstream effectors of phospho-Akt include the mammalian target of rapamycin (mTOR), glycogen synthase kinase 3, Bcl-2-associated death promoter, NF-κB, as well as, MAPK pathways signaling molecules, c-Jun NH2-terminal kinase (JNK) and extracellular signal-regulated kinase (ERK) (Wu et al., 2019). It has been demonstrated that phosphorylation of Akt at S473 (Akt1 pS473) in the carboxy-terminal hydrophobic motif, either by mTOR or by DNA-dependent protein kinase, stimulates full Akt activity(Hemmings and Restuccia, 2012). Furthermore, mTOR complex 1 (mTORC1) substrates involved in promoting protein synthesis and cellular proliferation are the eukaryotic translation initiation factor 4E binding protein 1 (4EBP1) and ribosomal protein S6 kinase (S6K1/RSK1), which phosphorylates the ribosomal protein S6 (S6/RPS6) (Hemmings and Restuccia, 2012). In the present study, the increased expression of the phosphorylated downstream effectors of PI3K/Akt/mTOR pathway and the Raf/MEK/ERK pathways, specifically, Akt1 phospho-S473 and RPS6 phospho-S235/236, as well as ERK1/2 phospho-Y204/197 and p90RSK phospho-S380 was found in ccRCC tumor tissue compared to corresponding non-tumor tissue. Based on this comprehensive analysis of phosphorylation status in downstream effectors of two important pro-survival pathways, we confirmed their constitutive activation in ccRCC.

We further have looked for the possible association of GSTO1-1 with some of these signaling molecules. GSTO1 co-immunoprecipitated with Akt (total/phosphorylated), phospho-RSK1p90 and phospho-RPS6, which clearly implies that the above-mentioned proteins might be the targets for GSTO1 deglutathionylase activity. Our results further imply a potential role of GSTO1-1 in regulating the activity of numerous other signaling molecules involved in cell death and survival. Recently, signaling events involving interaction of GSTO1 with type 1 ryanodine receptor, RyR1 has been implicated in a signaling pathway that stimulates cancer stem cell enrichment during chemotherapy (Lu et al., 2017). Lu et al. also reported increased GSTO1

expression in a HIF-dependent manner after exposure of breast cancer cells to chemotherapy. As consequence of GSTO1 interaction with RyR1, downstream PYK2/SRC/STAT3 signaling is activated (Lu et al., 2017). The study by Piaggi et al. associated the overexpression of GSTO1-1 with the protection against cisplatin-induced apoptosis. The proposed mechanism of drug resistance might be the activation of two survival pathways (Akt and ERK1/2), as well as inhibition of apoptotic pathway (JNK1) induced by overexpression of GSTO1-1 (Piaggi et al., 2010). Additionally, it has been shown that a deglutathionylation modification was involved in the activation of Akt (Liu et al., 2015). The potential regulation of Akt by GSTO1-1 might be of importance considering numerous downstream targets of Akt involved in various cellular processes, such as survival, growth, proliferation and metabolism (Hemmings and Restuccia, 2012).

Owing to its significant roles in cancer, several potential molecular targets of the PI3K/Akt signaling pathway have been proposed in cancer therapy (Wu et al., 2019). Until now, for metastatic ccRCC several targeted therapies have been designed, including targeted therapies against VEGF, such as sorafenib, sunitinib, pazopanib and axitinib (Hsieh et al., 2017b). In addition to their principal antitumor role, growing body of evidence shows that some of these compounds affect cellular redox homeostasis, by mostly favoring oxidative distress or even more reduced milieu (Teppo et al., 2017). Thus, multikinase inhibitors, such as sunitinib and sorafenib inhibit proliferation and angiogenesis by blocking vascular endothelial growth factor receptors (VEGFR-2 and VEGFR-3), platelet-derived growth factor receptor-beta (PDGFR beta) and RAF kinase, as well as affect redox state (Chiou et al., 2009). On one hand, sunitinib achieves antioxidant effects by both increasing GSH level and inhibiting neuronal nitric oxide synthase activity (NOS) (Cui et al., 2014; Thijs et al., 2015). On the other hand, sorafenib exhibits prooxidant effects by opposite mechanism, decreasing GSH pool (Chiou et al., 2009).

Considering implication of those targeted therapies in redox homeostasis, investigations of GSTO1 inhibitors in cancers could be beneficial. Recently, whole class of α-chloroacetamide-1, highly specific and highly sensitive inhibitors of GSTO1 that react irreversibly has been identified (Tsuboi et al., 2011, 2010). Several studies indicated promising results on antitumor effect of this class of inhibitors. Namely, in the

human breast cancer cell line, KT53 caused a significant increase in cisplatin-induced cell death (Tsuboi et al., 2011). So far, the most potent inhibitor of GSTO1-1 in the group of α-chloroacetamide compounds is proved to be C1-27 (Ramkumar et al., 2016). Indeed, C1-27 showed promising antitumor activity in both *in vitro* and *in vivo* models of colorectal cancer, without gross systemic toxicities (Ramkumar et al., 2016).

This study demonstrated that the concomitance of GSTO polymorphisms may influence ccRCC risk. Additionally, prognostic role has been shown only for *GSTO1* polymorphism. Furthermore, up-regulated GSTO1-1 and GSTO2-2 enzymes in ccRCC tumor tissue might contribute to aberrant redox homeostasis and tumor progression. The possible molecular mechanism underlying the role of GSTO1-1 in ccRCC progression might be partially explained by GSTO1-1 deglutathionylase activity.

6 CONCLUSIONS

- The results of this study showed that hypertension and smoking, as established risk factors, are associated with increased risk to clear cell renal cell carcinoma (ccRCC) occurrence.
    - Hypertensive subjects were at 3.54-fold higher risk of ccRCC development compared to normotensive subjects, while smokers exhibited 1.5-fold increased risk of ccRCC showing borderline significance.
- The polymorphisms in GSTO genes (*GSTO1* rs4925, *GSTO2* rs156697 and *GSTO2* rs2297235) significantly affect the risk to ccRCC, based on both gene-gene and gene-environment interactions.
    - Patients with ccRCC, carriers of combined variant *GSTO1* and *GSTO2* genotypes showed 2.6-fold higher risk of developing ccRCC in comparison with those one with combined referent genotypes.
    - The carriers of H2 haplotype, comprising all three variant alleles: *GSTO1**A (rs4925), *GSTO2**G (rs156697) and *GSTO2**G (rs2297235), exhibited the highest risk for ccRCC development compared to carriers of H1 haplotype, comprised of all three referent alleles.
    - In ccRCC patients, *GSTO2**G/G variant genotype (rs2297235) was significantly associated with higher oxidative DNA damage, measured as 8-OHdG levels.
    - Regarding the gene-environment interactions and ccRCC risk, smokers with variant *GSTO2* (rs156697) genotype were at higher risk in comparison with non-smokers carriers of at least one referent allele. Concerning other risk factors associated with ccRCC no modifying effect of GSTO genotypes was found in this study.
- GSTO1 polymorphism might have prognostic role, especially in male ccRCC patients.

- Shorter survival in male carriers of *GSTO1**C/C referent type genotype compared to the carriers of at least one variant allele was obtained.
- Moreover, *GSTO1**C/C referent genotype independently predicted higher risk of overall mortality among male ccRCC patients when the association between different GSTO genotypes and overall mortality, adjusted by recognized prognostic factors, was analyzed.
- No statistically significant results were obtained for investigated GSTO polymorphisms in terms of postoperative prognosis and the risk of overall mortality when the whole group, regardless of gender, was analyzed.

- The expression of both GSTO isoenzymes, GSTO1-1 and GSTO2/2, as well as, phosphorylated downstream effectors of PI3K/Akt and MAPK/ERK signaling pathways, known to be constitutively active, are up-regulated in ccRCC tumor in comparison to corresponding non-tumor tissue.
  - In addition to findings on both significant GSTO1-1 and GSTO2-2 upregulation in ccRCC when all patients were studied together, the change in expression levels of these two isoenzymes between early-stage and late-stage ccRCC was found.
  - The increased expression of the phosphorylated downstream effectors of PI3K/Akt/mTOR pathway and the Raf/MEK/ERK pathways was found in ccRCC tumor tissue compared to corresponding non-tumor tissue. Based on this comprehensive analysis of phosphorylation status in downstream effectors of two important pro-survival pathways, we confirmed their constitutive activation in ccRCC.
  - GSTO1 was co-immunoprecipitated with Akt (total/phosphorylated), phospho-RSK1p90 and phospho-RPS6.

The results of this study demonstrated that *GSTO1* and *GSTO2* polymorphisms play significant role in the risk and prognosis of ccRCC. Changes in GSTO1-1 expression might contribute to impaired redox homeostasis during ccRCC progression, which can be partially explained by its deglutathionylase activity.

7 REFERENCES

Ada, T.G., Ada, A.O., Kunak, S.C., Alpar, S., Gulhan, M., Iscan, M., 2013. Association between glutathione S-transferase omega 1 A140D polymorphism in the Turkish population and susceptibility to non-small cell lung cancer. Arh Hig Rada Toksikol 64, 61–67. https://doi.org/10.2478/10004-1254-64-2013-2302

Ahmad, S.T., Arjumand, W., Seth, A., Kumar Saini, A., Sultana, S., 2012. Impact of glutathione transferase M1, T1, and P1 gene polymorphisms in the genetic susceptibility of North Indian population to renal cell carcinoma. DNA Cell Biol. 31, 636–643. https://doi.org/10.1089/dna.2011.1392

Allen, M., Zou, F., Chai, H.S., Younkin, C.S., Miles, R., Nair, A.A., Crook, J.E., Pankratz, V.S., Carrasquillo, M.M., Rowley, C.N., Nguyen, T., Ma, L., Malphrus, K.G., Bisceglio, G., Ortolaza, A.I., Palusak, R., Middha, S., Maharjan, S., Georgescu, C., Schultz, D., Rakhshan, F., Kolbert, C.P., Jen, J., Sando, S.B., Aasly, J.O., Barcikowska, M., Uitti, R.J., Wszolek, Z.K., Ross, O.A., Petersen, R.C., Graff-Radford, N.R., Dickson, D.W., Younkin, S.G., Ertekin-Taner, N., 2012. Glutathione S-transferase omega genes in Alzheimer and Parkinson disease risk, age-at-diagnosis and brain gene expression: an association study with mechanistic implications. Mol Neurodegener 7, 13. https://doi.org/10.1186/1750-1326-7-13

An, J., Rettig, M.B., 2005. Mechanism of von Hippel-Lindau Protein-Mediated Suppression of Nuclear Factor kappa B Activity. Mol Cell Biol 25, 7546–7556. https://doi.org/10.1128/MCB.25.17.7546-7556.2005

Andonova, I.E., Justenhoven, C., Winter, S., Hamann, U., Baisch, C., Rabstein, S., Spickenheuer, A., Harth, V., Pesch, B., Brüning, T., Ko, Y.-D., Ganev, V., Brauch, H., 2010. No evidence for glutathione S-transferases GSTA2, GSTM2, GSTO1, GSTO2, and GSTZ1 in breast cancer risk. Breast Cancer Res. Treat. 121, 497–502. https://doi.org/10.1007/s10549-009-0589-5

Bamias, A., Escudier, B., Sternberg, C.N., Zagouri, F., Dellis, A., Djavan, B., Tzannis, K., Kontovinis, L., Stravodimos, K., Papatsoris, A., Mitropoulos, D., Deliveliotis, C., Dimopoulos, M., Constantinides, C.A., 2017. Current Clinical Practice Guidelines for the Treatment of Renal Cell Carcinoma: A Systematic Review and Critical Evaluation. The Oncologist 22, 667–679. https://doi.org/10.1634/theoncologist.2016-0435

Bartolini, D., Galli, F., 2016. The functional interactome of GSTP: A regulatory biomolecular network at the interface with the Nrf2 adaption response to oxidative stress. Journal of Chromatography B 1019, 29–44. https://doi.org/10.1016/j.jchromb.2016.02.002

Board, P.G., 2011. The omega-class glutathione transferases: structure, function, and genetics. Drug Metabolism Reviews 43, 226–235. https://doi.org/10.3109/03602532.2011.561353

Board, P.G., Anders, M.W., 2007. Glutathione Transferase Omega 1 Catalyzes the Reduction of S-(Phenacyl)glutathiones to Acetophenones. Chem Res Toxicol 20, 149–154. https://doi.org/10.1021/tx600305y

Board, P.G., Coggan, M., Chelvanayagam, G., Easteal, S., Jermiin, L.S., Schulte, G.K., Danley, D.E., Hoth, L.R., Griffor, M.C., Kamath, A.V., Rosner, M.H., Chrunyk, B.A., Perregaux, D.E., Gabel, C.A., Geoghegan, K.F., Pandit, J., 2000.

Identification, characterization, and crystal structure of the Omega class glutathione transferases. J. Biol. Chem. 275, 24798–24806. https://doi.org/10.1074/jbc.M001706200

Board, P.G., Menon, D., 2016. Structure, function and disease relevance of Omega-class glutathione transferases. Arch Toxicol 90, 1049–1067. https://doi.org/10.1007/s00204-016-1691-1

Board, P.G., Menon, D., 2013. Glutathione transferases, regulators of cellular metabolism and physiology. Biochimica et Biophysica Acta (BBA) - General Subjects 1830, 3267–3288. https://doi.org/10.1016/j.bbagen.2012.11.019

Braga, E.A., Fridman, M.V., Loginov, V.I., Dmitriev, A.A., Morozov, S.G., 2019. Molecular Mechanisms in Clear Cell Renal Cell Carcinoma: Role of miRNAs and Hypermethylated miRNA Genes in Crucial Oncogenic Pathways and Processes. Front. Genet. 10, 320. https://doi.org/10.3389/fgene.2019.00320

Bratslavsky, G., Sudarshan, S., Neckers, L., Linehan, W.M., 2007. Pseudohypoxic Pathways in Renal Cell Carcinoma. Clin Cancer Res 13, 4667–4671. https://doi.org/10.1158/1078-0432.CCR-06-2510

Buzio, L., De Palma, G., Mozzoni, P., Tondel, M., Buzio, C., Franchini, I., Axelson, O., Mutti, A., 2003. Glutathione S-transferases M1-1 and T1-1 as risk modifiers for renal cell cancer associated with occupational exposure to chemicals. Occup Environ Med 60, 789–793.

Cairns, R.A., Harris, I.S., Mak, T.W., 2011. Regulation of cancer cell metabolism. Nature Reviews Cancer 11, 85–95. https://doi.org/10.1038/nrc2981

Cancer Genome Atlas Research Network, 2013. Comprehensive molecular characterization of clear cell renal cell carcinoma. Nature 499, 43–49. https://doi.org/10.1038/nature12222

Chen, F., Zhang, Y., Şenbabaoğlu, Y., Ciriello, G., Yang, L., Reznik, E., Shuch, B., Micevic, G., De Velasco, G., Shinbrot, E., Noble, M.S., Lu, Y., Covington, K.R., Xi, L., Drummond, J.A., Muzny, D., Kang, H., Lee, J., Tamboli, P., Reuter, V., Shelley, C.S., Kaipparettu, B.A., Bottaro, D.P., Godwin, A.K., Gibbs, R.A., Getz, G., Kucherlapati, R., Park, P.J., Sander, C., Henske, E.P., Zhou, J.H., Kwiatkowski, D.J., Ho, T.H., Choueiri, T.K., Hsieh, J.J., Akbani, R., Mills, G.B., Hakimi, A.A., Wheeler, D.A., Creighton, C.J., 2016. Multilevel Genomics-Based Taxonomy of Renal Cell Carcinoma. Cell Rep 14, 2476–2489. https://doi.org/10.1016/j.celrep.2016.02.024

Chen, J.-H., Ni, R.-Z., Xiao, M.-B., Guo, J.-G., Zhou, J.-W., 2009. Comparative proteomic analysis of differentially expressed proteins in human pancreatic cancer tissue. HBPD INT 8, 193–200.

Chiou, J.-F., Tai, C.-J., Wang, Y.-H., Liu, T.-Z., Jen, Y.-M., Shiau, C.-Y., 2009. Sorafenib induces preferential apoptotic killing of a drug- and radio-resistant hep G2 cells through a mitochondria-dependent oxidative stress mechanism. Cancer Biology & Therapy 8, 1904–1913. https://doi.org/10.4161/cbt.8.20.9436

Chuang, M.-J., Sun, K.-H., Tang, S.-J., Deng, M.-W., Wu, Y.-H., Sung, J.-S., Cha, T.-L., Sun, G.-H., 2008. Tumor-derived tumor necrosis factor-alpha promotes progression and epithelial-mesenchymal transition in renal cell carcinoma cells. Cancer Science 99, 905–913. https://doi.org/10.1111/j.1349-7006.2008.00756.x

Coles, B.F., Kadlubar, F.F., 2005. Human Alpha Class Glutathione S-Transferases: Genetic Polymorphism, Expression, and Susceptibility to Disease, in: Methods

in Enzymology. Elsevier, pp. 9–42. https://doi.org/10.1016/S0076-6879(05)01002-5

Coll, R.C., O'Neill, L.A.J., 2011. The Cytokine Release Inhibitory Drug CRID3 Targets ASC Oligomerisation in the NLRP3 and AIM2 Inflammasomes. PLoS One 6. https://doi.org/10.1371/journal.pone.0029539

Cooper, A.J., Pinto, J.T., Callery, P.S., 2011. Reversible and irreversible protein glutathionylation: biological and clinical aspects. Expert Opinion on Drug Metabolism & Toxicology 7, 891–910. https://doi.org/10.1517/17425255.2011.577738

Coric, V.M., Simic, T.P., Pekmezovic, T.D., Basta-Jovanovic, G.M., Savic Radojevic, A.R., Radojevic-Skodric, S.M., Matic, M.G., Dragicevic, D.P., Radic, T.M., Bogdanovic, L.M., Dzamic, Z.M., Pljesa-Ercegovac, M.S., 2016. Combined GSTM1-Null, GSTT1-Active, GSTA1 Low-Activity and GSTP1-Variant Genotype Is Associated with Increased Risk of Clear Cell Renal Cell Carcinoma. PLoS ONE 11, e0160570. https://doi.org/10.1371/journal.pone.0160570

Cui, W., Zhang, Z.-J., Hu, S.-Q., Mak, S.-H., Xu, D.-P., Choi, C.-L., Wang, Y.-Q., Tsim, W.-K., Lee, M.-Y., Rong, J.-H., Han, Y.-F., 2014. Sunitinib Produces Neuroprotective Effect Via Inhibiting Nitric Oxide Overproduction. CNS Neurosci Ther 20, 244–252. https://doi.org/10.1111/cns.12203

Davis, C.F., Ricketts, C.J., Wang, M., Yang, Lixing, Cherniack, A.D., Shen, H., Buhay, C., Kang, H., Kim, S.C., Fahey, C.C., Hacker, K.E., Bhanot, G., Gordenin, D.A., Chu, A., Gunaratne, P.H., Biehl, M., Seth, S., Kaipparettu, B.A., Bristow, C.A., Donehower, L.A., Wallen, E.M., Smith, A.B., Tickoo, S.K., Tamboli, P., Reuter, V., Schmidt, L.S., Hsieh, J.J., Choueiri, T.K., Hakimi, A.A., Chin, L., Meyerson, M., Kucherlapati, R., Park, W.-Y., Robertson, A.G., Laird, P.W., Henske, E.P., Kwiatkowski, D.J., Park, P.J., Morgan, M., Shuch, B., Muzny, D., Wheeler, D.A., Linehan, W.M., Gibbs, R.A., Rathmell, W.K., Creighton, C.J., Creighton, C.J., Davis, C.F., Morgan, M., Gunaratne, P.H., Donehower, L.A., Kaipparettu, B.A., Wheeler, D.A., Gibbs, R.A., Signoretti, S., Cherniack, A.D., Robertson, A.G., Chu, A., Choueiri, T.K., Henske, E.P., Kwiatkowski, D.J., Reuter, V., Hsieh, J.J., Hakimi, A.A., Tickoo, S.K., Ricketts, C., Linehan, W.M., Schmidt, L.S., Gordenin, D.A., Bhanot, G., Seiler, M., Tamboli, P., Rathmell, W.K., Fahey, C.C., Hacker, K.E., Smith, A.B., Wallen, E.M., Shen, H., Laird, P.W., Shuch, B., Muzny, D., Buhay, C., Wang, M., Chao, H., Dahdouli, M., Xi, L., Kakkar, N., Reid, J.G., Downs, B., Drummond, J., Morton, D., Doddapaneni, H., Lewis, L., English, A., Meng, Q., Kovar, C., Wang, Q., Hale, W., Hawes, A., Kalra, D., Walker, K., Murray, B.A., Sougnez, C., Saksena, G., Carter, S.L., Schumacher, S.E., Tabak, B., Zack, T.I., Getz, G., Beroukhim, R., Gabriel, S.B., Meyerson, M., Ally, A., Balasundaram, M., Birol, I., Brooks, D., Butterfield, Y.S.N., Chuah, E., Clarke, A., Dhalla, N., Guin, R., Holt, R.A., Kasaian, K., Lee, D., Li, H.I., Lim, E., Ma, Y., Mayo, M., Moore, R.A., Mungall, A.J., Schein, J.E., Sipahimalani, P., Tam, A., Thiessen, N., Wong, T., Jones, S.J.M., Marra, M.A., Auman, J.T., Tan, D., Meng, S., Jones, C.D., Hoadley, K.A., Mieczkowski, P.A., Mose, L.E., Jefferys, S.R., Roach, J., Veluvolu, U., Wilkerson, M.D., Waring, S., Buda, E., Wu, J., Bodenheimer, T., Hoyle, A.P., Simons, J.V., Soloway, M.G., Balu, S., Parker, J.S., Hayes, D.N., Perou, C.M., Weisenberger, D.J., Bootwalla, M.S., Triche, T., Lai, P.H., Van Den Berg, D.J.,

Baylin, S.B., Chen, F., Coarfa, C., Noble, M.S., DiCara, D., Zhang, H., Cho, J., Heiman, D.I., Gehlenborg, N., Voet, D., Lin, P., Frazer, S., Stojanov, P., Liu, Y., Zou, L., Kim, J., Lawrence, M.S., Chin, L., Yang, Lixing, Seth, S., Bristow, C.A., Protopopov, A., Song, X., Zhang, Jianhua, Pantazi, A., Hadjipanayis, A., Lee, E., Luquette, L.J., Lee, S., Parfenov, M., Santoso, N., Seidman, J., Xu, A.W., Kucherlapati, R., Park, P.J., Kang, H., Lee, J., Roberts, S.A., Klimczak, L.J., Fargo, D., Lang, M., Choi, Y.-L., Kim, S.C., Lee, J.-K., Park, W.-Y., Wang, W., Fan, Y., Ahn, J., Akbani, R., Weinstein, J.N., Haussler, D., Ma, S., Radenbaugh, A., Zhul, J., Biehl, M., Lichtenberg, T.M., Zmuda, E., Black, A.D., Hanf, B., Ramirez, N.C., Wise, L., Bowen, J., Leraas, K.M., Hall, T.M., Gastier-Foster, J.M., Kaelin, W.G., Thorne, L., Boice, L., Huang, M., Vocke, C., Peterson, J., Worrell, R., Merino, M., Czerniak, B.A., Aldape, K.D., Wood, C.G., Spellman, P.T., Atkins, M.B., Cheville, J., Thompson, R.H., Jensen, M.A., Pihl, T., Wan, Y., Ayala, B., Baboud, J., Velaga, S., Walton, J., Liu, J., Chudamani, S., Wu, Y., Sheth, M., Mills Shaw, K.R., Demchok, J.A., Davidsen, T., Yang, Liming, Wang, Z., Tarnuzzer, R.W., Zhang, Jiashan, Eley, G., Felau, I., Zenklusen, J.C., Hutter, C.M., Guyer, M.S., Ozenberger, B.A., Sofia, H.J., 2014. The Somatic Genomic Landscape of Chromophobe Renal Cell Carcinoma. Cancer Cell 26, 319–330. https://doi.org/10.1016/j.ccr.2014.07.014

De Martino, M., Klatte, T., Schatzl, G., Remzi, M., Waldert, M., Haitel, A., Stancik, I., Kramer, G., Marberger, M., 2010. Renal cell carcinoma Fuhrman grade and histological subtype correlate with complete polymorphic deletion of glutathione S-transferase M1 gene. J. Urol. 183, 878–883. https://doi.org/10.1016/j.juro.2009.11.032

Di Pietro, G., Magno, L.A.V., Rios-Santos, F., 2010. Glutathione S-transferases: an overview in cancer research. Expert Opin Drug Metab Toxicol 6, 153–170. https://doi.org/10.1517/17425250903427980

Djukic, T., Simic, T., Pljesa-Ercegovac, M., Matic, M., Suvakov, S., Coric, V., Dragicevic, D., Savic-Radojevic, A., 2017. Upregulated glutathione transferase omega-1 correlates with progression of urinary bladder carcinoma. Redox Report 22, 486–492. https://doi.org/10.1080/13510002.2017.1299909

Djukic, T., Simic, T., Radic, T., Matic, M., Pljesa-Ercegovac, M., Suvakov, S., Coric, V., Pekmezovic, T., Novakovic, I., Dragicevic, D., Savic-Radojevic, A., 2015. GSTO1*C/GSTO2*G haplotype is associated with risk of transitional cell carcinoma of urinary bladder. International Urology and Nephrology 47, 625–630. https://doi.org/10.1007/s11255-015-0933-0

Djukic, T.I., Savic-Radojevic, A.R., Pekmezovic, T.D., Matic, M.G., Pljesa-Ercegovac, M.S., Coric, V.M., Radic, T.M., Suvakov, S.R., Krivic, B.N., Dragicevic, D.P., Simic, T.P., 2013. Glutathione S-transferase T1, O1 and O2 polymorphisms are associated with survival in muscle invasive bladder cancer patients. PLoS ONE 8, e74724. https://doi.org/10.1371/journal.pone.0074724

Dulhunty, A., Gage, P., Curtis, S., Chelvanayagam, G., Board, P., 2001. The Glutathione Transferase Structural Family Includes a Nuclear Chloride Channel and a Ryanodine Receptor Calcium Release Channel Modulator. J. Biol. Chem. 276, 3319–3323. https://doi.org/10.1074/jbc.M007874200

Dulhunty, A.F., Haarmann, C.S., Green, D., Laver, D.R., Board, P.G., Casarotto, M.G., 2002. Interactions between dihydropyridine receptors and ryanodine receptors in

striated muscle. Progress in Biophysics and Molecular Biology 79, 45–75. https://doi.org/10.1016/S0079-6107(02)00013-5

Dulhunty, A.F., Hewawasam, R., Liu, D., Casarotto, M.G., Board, P.G., 2011. Regulation of the cardiac muscle ryanodine receptor by glutathione transferases. Drug Metabolism Reviews 43, 236–252. https://doi.org/10.3109/03602532.2010.549134

Eble, J.N., Weltgesundheitsorganisation, International Agency for Research on Cancer (Eds.), 2006. Pathology and genetics of tumours of the urinary system and male genital organs: ... editorial and consensus conference in Lyon, France, December 14 - 18, 2002, Reprint. ed, World Health Organization classification of tumours. IARC Press, Lyon.

Escudier, B., Porta, C., Schmidinger, M., Algaba, F., Patard, J.J., Khoo, V., Eisen, T., Horwich, A., on behalf of the ESMO Guidelines Working Group, 2014. Renal cell carcinoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of Oncology 25, iii49–iii56. https://doi.org/10.1093/annonc/mdu259

Fabrizio, F.P., Costantini, M., Copetti, M., la Torre, A., Sparaneo, A., Fontana, A., Poeta, L., Gallucci, M., Sentinelli, S., Graziano, P., Parente, P., Pompeo, V., Salvo, L.D., Simone, G., Papalia, R., Picardo, F., Balsamo, T., Flammia, G.P., Trombetta, D., Pantalone, A., Kok, K., Paranita, F., Muscarella, L.A., Fazio, V.M., 2017. Keap1/Nrf2 pathway in kidney cancer: frequent methylation of KEAP1 gene promoter in clear renal cell carcinoma. Oncotarget 8, 11187–11198. https://doi.org/10.18632/oncotarget.14492

Frei, B., England, L., Ames, B.N., 1989. Ascorbate is an outstanding antioxidant in human blood plasma. Proceedings of the National Academy of Sciences 86, 6377–6381. https://doi.org/10.1073/pnas.86.16.6377

Gago-Dominguez, M., Castelao, J.E., Yuan, J.-M., Ross, R.K., Yu, M.C., 2002. Lipid peroxidation: a novel and unifying concept of the etiology of renal cell carcinoma (United States). Cancer Causes Control 13, 287–293.

Gao, Y.H., Li, C.X., Shen, S.M., Li, H., Chen, G.Q., Wei, Q., Wang, L.S., 2013. Hypoxia-inducible factor 1α mediates the down-regulation of superoxide dismutase 2 in von Hippel-Lindau deficient renal clear cell carcinoma. Biochem Biophys Res Commun 435, 46–51. https://doi.org/10.1016/j.bbrc.2013.04.034

Gill, I.S., Aron, M., Gervais, D.A., Jewett, M.A.S., 2010. Clinical practice. Small renal mass. N. Engl. J. Med. 362, 624–634. https://doi.org/10.1056/NEJMcp0910041

Guengerich, F.P., 2005. Activation of alkyl halides by glutathione transferases. Meth. Enzymol. 401, 342–353. https://doi.org/10.1016/S0076-6879(05)01021-9

Guo, H., German, P., Bai, S., Barnes, S., Guo, W., Qi, X., Lou, H., Liang, J., Jonasch, E., Mills, G.B., Ding, Z., 2015. The PI3K/AKT Pathway and Renal Cell Carcinoma. Journal of Genetics and Genomics 42, 343–353. https://doi.org/10.1016/j.jgg.2015.03.003

Györke, S., 2009. Molecular basis of catecholaminergic polymorphic ventricular tachycardia. Heart Rhythm 6, 123–129. https://doi.org/10.1016/j.hrthm.2008.09.013

Haas, N.B., Nathanson, K.L., 2014. Hereditary kidney cancer syndromes. Adv Chronic Kidney Dis 21, 81–90. https://doi.org/10.1053/j.ackd.2013.10.001

Haddad, A.Q., Margulis, V., 2015. Tumour and patient factors in renal cell carcinoma-towards personalized therapy. Nat Rev Urol 12, 253–262. https://doi.org/10.1038/nrurol.2015.71

Hakimi, A.A., Chen, Y.-B., Wren, J., Gonen, M., Abdel-Wahab, O., Heguy, A., Liu, H., Takeda, S., Tickoo, S.K., Reuter, V.E., Voss, M.H., Motzer, R.J., Coleman, J.A., Cheng, E.H., Russo, P., Hsieh, J.J., 2013a. Clinical and Pathologic Impact of Select Chromatin Modulating Tumor Suppressors in Clear Cell Renal Cell Carcinoma. Eur Urol 63, 848–854. https://doi.org/10.1016/j.eururo.2012.09.005

Hakimi, A.A., Ostrovnaya, I., Reva, B., Schultz, N., Chen, Y.-B., Gonen, M., Liu, H., Takeda, S., Voss, M.H., Tickoo, S.K., Reuter, V.E., Russo, P., Cheng, E.H., Sander, C., Motzer, R.J., Hsieh, J.J., 2013b. Adverse Outcomes in Clear Cell Renal Cell Carcinoma with Mutations of 3p21 Epigenetic Regulators BAP1 and SETD2: a Report by MSKCC and the KIRC TCGA Research Network. Clin Cancer Res 19, 3259–3267. https://doi.org/10.1158/1078-0432.CCR-12-3886

Hakimi, A.A., Reznik, E., Lee, C.-H., Creighton, C.J., Brannon, A.R., Luna, A., Aksoy, B.A., Liu, E.M., Shen, R., Lee, W., Chen, Y., Stirdivant, S.M., Russo, P., Chen, Y.B., Tickoo, S.K., Reuter, V.E., Cheng, E.H., Sander, C., Hsieh, J.J., 2016. An Integrated Metabolic Atlas of Clear Cell Renal Cell Carcinoma. Cancer Cell 29, 104–116. https://doi.org/10.1016/j.ccell.2015.12.004

Harju, T.H., Peltoniemi, M.J., Rytilä, P.H., Soini, Y., Salmenkivi, K.M., Board, P.G., Ruddock, L.W., Kinnula, V.L., 2007. Glutathione S-transferase omega in the lung and sputum supernatants of COPD patients. Respir Res 8, 48. https://doi.org/10.1186/1465-9921-8-48

Hartl, D.L., Clark, A.G., 2007. Principles of population genetics, 4th ed. ed. Sinauer Associates, Sunderland, Mass.

Hayes, J.D., Flanagan, J.U., Jowsey, I.R., 2005. Glutathione transferases. Annu. Rev. Pharmacol. Toxicol. 45, 51–88. https://doi.org/10.1146/annurev.pharmtox.45.120403.095857

Hayes, J.D., McLellan, L.I., 1999. Glutathione and glutathione-dependent enzymes represent a co-ordinately regulated defence against oxidative stress. Free Radic. Res. 31, 273–300.

Hayes, J.D., Pulford, D.J., 1995. The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. Crit. Rev. Biochem. Mol. Biol. 30, 445–600. https://doi.org/10.3109/10409239509083491

Hedrick, P.W., 2011. Genetics of populations, 4th ed. ed. Jones and Bartlett Publishers, Sudbury, Mass.

Hemmings, B.A., Restuccia, D.F., 2012. PI3K-PKB/Akt Pathway. Cold Spring Harbor Perspectives in Biology 4, a011189–a011189. https://doi.org/10.1101/cshperspect.a011189

Hollman, A., Tchounwou, P., Huang, H.-C., 2016. The Association between Gene-Environment Interactions and Diseases Involving the Human GST Superfamily with SNP Variants. International Journal of Environmental Research and Public Health 13, 379. https://doi.org/10.3390/ijerph13040379

Hsieh, J.J., Manley, B.J., Khan, N., Gao, J., Carlo, M.I., Cheng, E.H., 2017a. Overcome tumor heterogeneity-imposed therapeutic barriers through convergent genomic biomarker discovery: A braided cancer river model of kidney cancer. Semin. Cell Dev. Biol. 64, 98–106. https://doi.org/10.1016/j.semcdb.2016.09.002

Hsieh, J.J., Purdue, M.P., Signoretti, S., Swanton, C., Albiges, L., Schmidinger, M., Heng, D.Y., Larkin, J., Ficarra, V., 2017b. Renal cell carcinoma. Nat Rev Dis Primers 3, 17009. https://doi.org/10.1038/nrdp.2017.9

Ikemoto, S., Yoshida, N., Narita, K., Wada, S., Kishimoto, T., Sugimura, K., Nakatani, T., 2003. Role of tumor-associated macrophages in renal cell carcinoma. Oncol. Rep. 10, 1843–1849.

Jahangiri, A., Weiss, W.A., 2013. It Takes Two to Tango: Dual Inhibition of PI3K and MAPK in Rhabdomyosarcoma. Clinical Cancer Research 19, 5811–5813. https://doi.org/10.1158/1078-0432.CCR-13-2177

Karami, S., Boffetta, P., Rothman, N., Hung, R.J., Stewart, T., Zaridze, D., Navritalova, M., Mates, D., Janout, V., Kollarova, H., Bencko, V., Szeszenia-Dabrowska, N., Holcatova, I., Mukeria, A., Gromiec, J., Chanock, S.J., Brennan, P., Chow, W.-H., Moore, L.E., 2008. Renal cell carcinoma, occupational pesticide exposure and modification by glutathione S-transferase polymorphisms. Carcinogenesis 29, 1567–1571. https://doi.org/10.1093/carcin/bgn153

Kölsch, H., Larionov, S., Dedeck, O., Orantes, M., Birkenmeier, G., Griffin, W.S.T., Thal, D.R., 2007. Association of the glutathione S-transferase omega-1 Ala140Asp polymorphism with cerebrovascular atherosclerosis and plaque-associated interleukin-1 alpha expression. Stroke 38, 2847–2850. https://doi.org/10.1161/STROKEAHA.107.484162

Kumar, A., Kumari, N., Gupta, V., Prasad, R., 2018. Renal Cell Carcinoma: Molecular Aspects. Indian J Clin Biochem 33, 246–254. https://doi.org/10.1007/s12291-017-0713-y

Kurtovic, S., Grehn, L., Karlsson, A., Hellman, U., Mannervik, B., 2008. Glutathione transferase activity with a novel substrate mimics the activation of the prodrug azathioprine. Anal. Biochem. 375, 339–344. https://doi.org/10.1016/j.ab.2007.12.033

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680–685.

Laliberte, R.E., Perregaux, D.G., Hoth, L.R., Rosner, P.J., Jordan, C.K., Peese, K.M., Eggler, J.F., Dombroski, M.A., Geoghegan, K.F., Gabel, C.A., 2003. Glutathione S-Transferase Omega 1-1 Is a Target of Cytokine Release Inhibitory Drugs and May Be Responsible for Their Effect on Interleukin-1β Posttranslational Processing. J. Biol. Chem. 278, 16567–16578. https://doi.org/10.1074/jbc.M211596200

Lanner, J.T., Georgiou, D.K., Joshi, A.D., Hamilton, S.L., 2010. Ryanodine Receptors: Structure, Expression, Molecular Details, and Function in Calcium Release. Cold Spring Harb Perspect Biol 2. https://doi.org/10.1101/cshperspect.a003996

Laurent, A., Nicco, C., Chéreau, C., Goulvestre, C., Alexandre, J., Alves, A., Lévy, E., Goldwasser, F., Panis, Y., Soubrane, O., Weill, B., Batteux, F., 2005. Controlling tumor growth by modulating endogenous production of reactive oxygen species. Cancer Res. 65, 948–956.

Lei, K., Townsend, D.M., Tew, K.D., 2008. Protein cysteine sulfinic acid reductase (sulfiredoxin) as a regulator of cell proliferation and drug response. Oncogene 27, 4877–4887. https://doi.org/10.1038/onc.2008.132

Lesseur, C., Gilbert-Diamond, D., Andrew, A.S., M. Ekstrom, R., Li, Z., Kelsey, K.T., Marsit, C.J., Karagas, M.R., 2012. A case-control study of polymorphisms in xenobiotic and arsenic metabolism genes and arsenic-related bladder cancer in

New Hampshire. Toxicology Letters 210, 100–106. https://doi.org/10.1016/j.toxlet.2012.01.015

Li, P., Wu, M., Wang, J., Sui, Y., Liu, S., Shi, D., 2016. NAC selectively inhibit cancer telomerase activity: A higher redox homeostasis threshold exists in cancer cells. Redox Biology 8, 91–97. https://doi.org/10.1016/j.redox.2015.12.001

Li, Q.K., Pavlovich, C.P., Zhang, H., Kinsinger, C.R., Chan, D.W., 2019. Challenges and opportunities in the proteomic characterization of clear cell renal cell carcinoma (ccRCC): A critical step towards the personalized care of renal cancers. Seminars in Cancer Biology, Translational Cancer Genomics 55, 8–15. https://doi.org/10.1016/j.semcancer.2018.06.004

Li, Y., Schellhorn, H.E., 2007. New developments and novel therapeutic perspectives for vitamin C. J. Nutr. 137, 2171–2184.

Li, Y., Zhang, Q., Peng, B., Shao, Q., Qian, W., Zhang, J.-Y., 2014. Identification of glutathione S-transferase omega 1 (GSTO1) protein as a novel tumor-associated antigen and its autoantibody in human esophageal squamous cell carcinoma. Tumor Biology 35, 10871–10877. https://doi.org/10.1007/s13277-014-2394-y

Lindahl, M., Mata-Cabana, A., Kieselbach, T., 2011. The Disulfide Proteome and Other Reactive Cysteine Proteomes: Analysis and Functional Significance. Antioxidants & Redox Signaling 14, 2581–2642. https://doi.org/10.1089/ars.2010.3551

Lipworth, L., Tarone, R.E., Lund, L., McLaughlin, J.K., 2009. Epidemiologic characteristics and risk factors for renal cell cancer. Clin Epidemiol 1, 33–43.

Liu, X., Jann, J., Xavier, C., Wu, H., 2015. Glutaredoxin 1 (Grx1) Protects Human Retinal Pigment Epithelial Cells From Oxidative Damage by Preventing AKT Glutathionylation. Invest. Ophthalmol. Vis. Sci. 56, 2821. https://doi.org/10.1167/iovs.14-15876

Lo, H.-W., Ali-Osman, F., 2007. Genetic polymorphism and function of glutathione S-transferases in tumor drug resistance. Current Opinion in Pharmacology, Cancer/Immunomodulation 7, 367–374. https://doi.org/10.1016/j.coph.2007.06.009

Lombardi, S., Fuoco, I., di Fluri, G., Costa, F., Ricchiuti, A., Biondi, G., Nardini, V., Scarpato, R., 2015. Genomic instability and cellular stress in organ biopsies and peripheral blood lymphocytes from patients with colorectal cancer and predisposing pathologies. Oncotarget 6. https://doi.org/10.18632/oncotarget.4032

Lu, H., Chen, I., Shimoda, L.A., Park, Y., Zhang, C., Tran, L., Zhang, H., Semenza, G.L., 2017. Chemotherapy-Induced Ca2+ Release Stimulates Breast Cancer Stem Cell Enrichment. Cell Reports 18, 1946–1957. https://doi.org/10.1016/j.celrep.2017.02.001

Lusini, L., Tripodi, S.A., Rossi, R., Giannerini, F., Giustarini, D., del Vecchio, M.T., Barbanti, G., Cintorino, M., Tosi, P., Di Simplicio, P., 2001. Altered glutathione anti-oxidant metabolism during tumor progression in human renal-cell carcinoma. Int. J. Cancer 91, 55–59.

Marahatta, S.B., Punyarit, P., Bhudisawasdi, V., Paupairoj, A., Wongkham, S., Petmitr, S., 2006. Polymorphism of glutathione S-transferase Omega gene and risk of cancer. Cancer Letters 236, 276–281. https://doi.org/10.1016/j.canlet.2005.05.020

Masoudi, M., Saadat, I., Omidvari, S., Saadat, M., 2011. Association between N142D genetic polymorphism of GSTO2 and susceptibility to colorectal cancer. Mol Biol Rep 38, 4309–4313. https://doi.org/10.1007/s11033-010-0555-7

Masoudi, M., Saadat, I., Omidvari, S., Saadat, M., 2009. Genetic polymorphisms of GSTO2, GSTM1, and GSTT1 and risk of gastric cancer. Mol Biol Rep 36, 781–784. https://doi.org/10.1007/s11033-008-9245-0

Masson, N., Ratcliffe, P.J., 2014. Hypoxia signaling pathways in cancer metabolism: the importance of co-selecting interconnected physiological pathways. Cancer Metab 2, 3. https://doi.org/10.1186/2049-3002-2-3

Mehdi, A., Riazalhosseini, Y., 2017. Epigenome Aberrations: Emerging Driving Factors of the Clear Cell Renal Cell Carcinoma. Int J Mol Sci 18. https://doi.org/10.3390/ijms18081774

Menon, D., Board, P.G., 2013. A Role for Glutathione Transferase Omega 1 (GSTO1-1) in the Glutathionylation Cycle. Journal of Biological Chemistry 288, 25769–25779. https://doi.org/10.1074/jbc.M113.487785

Menon, D., Coll, R., O′Neill, L.A.J., Board, P.G., 2014. Glutathione transferase Omega 1 is required for the lipopolysaccharide-stimulated induction of NADPH oxidase 1 and the production of reactive oxygen species in macrophages. Free Radical Biology and Medicine 73, 318–327. https://doi.org/10.1016/j.freeradbiomed.2014.05.020

Mieyal, J.J., Gallogly, M.M., Qanungo, S., Sabens, E.A., Shelton, M.D., 2008. Molecular Mechanisms and Clinical Implications of Reversible Protein *S* -Glutathionylation. Antioxidants & Redox Signaling 10, 1941–1988. https://doi.org/10.1089/ars.2008.2089

Mukherjee, B., Salavaggione, O.E., Pelleymounter, L.L., Moon, I., Eckloff, B.W., Schaid, D.J., Wieben, E.D., Weinshilboum, R.M., 2006. Glutathione S-transferase omega 1 and omega 2 pharmacogenomics. Drug Metabolism and Disposition 34, 1237–1246. https://doi.org/10.1124/dmd.106.009613

Nam, S.J., Lee, C., Park, J.H., Moon, K.C., 2015. Decreased PBRM1 expression predicts unfavorable prognosis in patients with clear cell renal cell carcinoma. Urologic Oncology: Seminars and Original Investigations, Advances in Relapsed and Refractory Germ Cell Tumors 33, 340.e9-340.e16. https://doi.org/10.1016/j.urolonc.2015.01.010

Németi, B., Poór, M., Gregus, Z., 2015. Reduction of the Pentavalent Arsenical Dimethylarsinic Acid and the GSTO1 Substrate *S* -(4-Nitrophenacyl)glutathione by Rat Liver Cytosol: Analyzing the Role of GSTO1 in Arsenic Reduction. Chem. Res. Toxicol. 28, 2199–2209. https://doi.org/10.1021/acs.chemrestox.5b00368

Olsen, A., Autrup, H., Sørensen, M., Overvad, K., Tjønneland, A., 2008. Polymorphisms of glutathione S-transferase A1 and O1 and breast cancer among postmenopausal Danish women. Eur. J. Cancer Prev. 17, 225–229. https://doi.org/10.1097/CEJ.0b013e3282b6fe1e

Pace, N.J., Pimental, D.R., Weerapana, E., 2012. An inhibitor of glutathione S-transferase omega 1 that selectively targets apoptotic cells. Angew. Chem. Int. Ed. Engl. 51, 8365–8368. https://doi.org/10.1002/anie.201203730

Peña-Llopis, S., Vega-Rubín-de-Celis, S., Liao, A., Leng, N., Pavía-Jiménez, A., Wang, S., Yamasaki, T., Zhrebker, L., Sivanand, S., Spence, P., Kinch, L., Hambuch, T., Jain, S., Lotan, Y., Margulis, V., Sagalowsky, A.I., Summerour, P.B.,

Kabbani, W., Wong, S.W.W., Grishin, N., Laurent, M., Xie, X.-J., Haudenschild, C.D., Ross, M.T., Bentley, D.R., Kapur, P., Brugarolas, J., 2012. BAP1 loss defines a new class of renal cell carcinoma. Nat Genet 44, 751–759. https://doi.org/10.1038/ng.2323

Petrella, B.L., Vincenti, M.P., 2012. Interleukin-1β mediates metalloproteinase-dependent renal cell carcinoma tumor cell invasion through the activation of CCAAT enhancer binding protein β. Cancer Med 1, 17–27. https://doi.org/10.1002/cam4.7

Piacentini, S., Monaci, P.M., Polimanti, R., Manfellotto, D., Fuciarelli, M., 2013. GSTO2*N142D gene polymorphism associated with hypothyroidism in Italian patients. Mol Biol Rep 40, 1967–1971. https://doi.org/10.1007/s11033-012-2253-0

Piaggi, S., Marchi, S., Ciancia, E., DeBortoli, N., Lazzarotti, A., Saviozzi, M., Raggi, C., Fierabracci, V., Visvikis, A., Bisgaard, H.C., Casini, A.F., Paolicchi, A., 2009. Nuclear translocation of glutathione transferase omega is a progression marker in Barrett's esophagus. Oncology Reports 21, 283–287.

Piaggi, S., Raggi, C., Corti, A., Pitzalis, E., Mascherpa, M.C., Saviozzi, M., Pompella, A., Casini, A.F., 2010. Glutathione transferase omega 1-1 (GSTO1-1) plays an anti-apoptotic role in cell resistance to cisplatin toxicity. Carcinogenesis 31, 804–811. https://doi.org/10.1093/carcin/bgq031

Pljesa-Ercegovac, M., Mimic-Oka, J., Dragicevic, D., Savic-Radojevic, A., Opacic, M., Pljesa, S., Radosavljevic, R., Simic, T., 2008. Altered antioxidant capacity in human renal cell carcinoma: role of glutathione associated enzymes. Urol. Oncol. 26, 175–181. https://doi.org/10.1016/j.urolonc.2007.02.007

Pljesa-Ercegovac, M., Savic-Radojevic, A., Coric, V., Radic, T., Simic, T., 2019. Glutathione transferase genotypes may serve as determinants of risk and prognosis in renal cell carcinoma. BioFactors. https://doi.org/10.1002/biof.1560

Pljesa-Ercegovac, M., Savic-Radojevic, A., Matic, M., Coric, V., Djukic, T., Radic, T., Simic, T., 2018. Glutathione Transferases: Potential Targets to Overcome Chemoresistance in Solid Tumors. Int J Mol Sci 19. https://doi.org/10.3390/ijms19123785

Pongstaporn, W., Pakakasama, S., Sanguansin, S., Hongeng, S., Petmitr, S., 2009. Polymorphism of glutathione S-transferase Omega gene: association with risk of childhood acute lymphoblastic leukemia. J Cancer Res Clin Oncol 135, 673–678. https://doi.org/10.1007/s00432-008-0501-4

Pongstaporn, W., Rochanawutanon, M., Wilailak, S., Linasamita, V., Weerakiat, S., Petmitr, S., 2006. Genetic alterations in chromosome 10q24.3 and glutathione S-transferase omega 2 gene polymorphism in ovarian cancer. J. Exp. Clin. Cancer Res. 25, 107–114.

Purdue, M.P., Johansson, M., Zelenika, D., Toro, J.R., Scelo, G., Moore, L.E., Prokhortchouk, E., Wu, X., Kiemeney, L.A., Gaborieau, V., Jacobs, K.B., Chow, W.-H., Zaridze, D., Matveev, V., Lubinski, J., Trubicka, J., Szeszenia-Dabrowska, N., Lissowska, J., Rudnai, P., Fabianova, E., Bucur, A., Bencko, V., Foretova, L., Janout, V., Boffetta, P., Colt, J.S., Davis, F.G., Schwartz, K.L., Banks, R.E., Selby, P.J., Harnden, P., Berg, C.D., Hsing, A.W., Grubb, R.L., Boeing, H., Vineis, P., Clavel-Chapelon, F., Palli, D., Tumino, R., Krogh, V., Panico, S., Duell, E.J., Quirós, J.R., Sanchez, M.-J., Navarro, C., Ardanaz, E., Dorronsoro, M., Khaw, K.-T., Allen, N.E., Bueno-de-Mesquita, H.B., Peeters,

P.H.M., Trichopoulos, D., Linseisen, J., Ljungberg, B., Overvad, K., Tjønneland, A., Romieu, I., Riboli, E., Mukeria, A., Shangina, O., Stevens, V.L., Thun, M.J., Diver, W.R., Gapstur, S.M., Pharoah, P.D., Easton, D.F., Albanes, D., Weinstein, S.J., Virtamo, J., Vatten, L., Hveem, K., Njølstad, I., Tell, G.S., Stoltenberg, C., Kumar, R., Koppova, K., Cussenot, O., Benhamou, S., Oosterwijk, E., Vermeulen, S.H., Aben, K.K.H., van der Marel, S.L., Ye, Y., Wood, C.G., Pu, X., Mazur, A.M., Boulygina, E.S., Chekanov, N.N., Foglio, M., Lechner, D., Gut, I., Heath, S., Blanche, H., Hutchinson, A., Thomas, G., Wang, Z., Yeager, M., Fraumeni, J.F., Skryabin, K.G., McKay, J.D., Rothman, N., Chanock, S.J., Lathrop, M., Brennan, P., 2011. Genome-wide association study of renal cell carcinoma identifies two susceptibility loci on 2p21 and 11q13.3. Nat. Genet. 43, 60–65. https://doi.org/10.1038/ng.723

Ramkumar, K., Samanta, S., Kyani, A., Yang, S., Tamura, S., Ziemke, E., Stuckey, J.A., Li, S., Chinnaswamy, K., Otake, H., Debnath, B., Yarovenko, V., Sebolt-Leopold, J.S., Ljungman, M., Neamati, N., 2016. Mechanistic evaluation and transcriptional signature of a glutathione S-transferase omega 1 inhibitor. Nat Commun 7. https://doi.org/10.1038/ncomms13084

Rini, B.I., Campbell, S.C., Escudier, B., 2009. Renal cell carcinoma 373, 14.

Rodic, S., Vincent, M.D., 2018. Reactive oxygen species (ROS) are a key determinant of cancer's metabolic phenotype: ROS are a key determinant of cancer's metabolic phenotype. Int. J. Cancer 142, 440–448. https://doi.org/10.1002/ijc.31069

Sanchez, D.J., Simon, M.C., 2018. Genetic and metabolic hallmarks of clear cell renal cell carcinoma. Biochim Biophys Acta Rev Cancer 1870, 23–31. https://doi.org/10.1016/j.bbcan.2018.06.003

Sanguansin, S., Petmitr, S., O-charoenrat, P., Pongstaporn, W., 2012. Association of glutathione S-transferase omega gene polymorphisms with progression of head and neck cancer. Mol Biol Rep 39, 10915–10920. https://doi.org/10.1007/s11033-012-1991-3

Sato, Y., Yoshizato, T., Shiraishi, Y., Maekawa, S., Okuno, Y., Kamura, T., Shimamura, T., Sato-Otsubo, A., Nagae, G., Suzuki, H., Nagata, Y., Yoshida, K., Kon, A., Suzuki, Y., Chiba, K., Tanaka, H., Niida, A., Fujimoto, A., Tsunoda, T., Morikawa, T., Maeda, D., Kume, H., Sugano, S., Fukayama, M., Aburatani, H., Sanada, M., Miyano, S., Homma, Y., Ogawa, S., 2013. Integrated molecular analysis of clear-cell renal cell carcinoma. Nat. Genet. 45, 860–867. https://doi.org/10.1038/ng.2699

Savic-Radojevic, A., Djukic, T., Simic, T., Pljesa-Ercegovac, M., Dragicevic, D., Pekmezovic, T., Cekerevac, M., Santric, V., Matic, M., 2013. GSTM1-null and GSTA1-low activity genotypes are associated with enhanced oxidative damage in bladder cancer. Redox Report 18, 1–7. https://doi.org/10.1179/1351000212Y.0000000031

Schmuck, E.M., Board, P.G., Whitbread, A.K., Tetlow, N., Cavanaugh, J.A., Blackburn, A.C., Masoumi, A., 2005. Characterization of the monomethylarsonate reductase and dehydroascorbate reductase activities of Omega class glutathione transferase variants: implications for arsenic metabolism and the age-at-onset of Alzheimer's and Parkinson's diseases. Pharmacogenet. Genomics 15, 493–501.

Schödel, J., Bardella, C., Sciesielski, L.K., Brown, J.M., Pugh, C.W., Buckle, V., Tomlinson, I.P., Ratcliffe, P.J., Mole, D.R., 2012. Common genetic variants at

the 11q13.3 renal cancer susceptibility locus influence binding of HIF to an enhancer of cyclin D1 expression. Nat. Genet. 44, 420–425, S1-2. https://doi.org/10.1038/ng.2204

Searchfield, L., Price, S.A., Betton, G., Jasani, B., Riccardi, D., Griffiths, D.F.R., 2011. Glutathione S-transferases as molecular markers of tumour progression and prognosis in renal cell carcinoma: GST-alpha in human RCC. Histopathology 58, 180–190. https://doi.org/10.1111/j.1365-2559.2010.03733.x

Semenza, G.L., 2013. HIF-1 mediates metabolic responses to intratumoral hypoxia and oncogenic mutations. J Clin Invest 123, 3664–3671. https://doi.org/10.1172/JCI67230

Slatkin, M., 2008. Linkage disequilibrium--understanding the evolutionary past and mapping the medical future. Nat. Rev. Genet. 9, 477–485. https://doi.org/10.1038/nrg2361

Sobin, L.H., Gospodarowicz, M.K., Wittekind, C., International Union against Cancer (Eds.), 2010. TNM classification of malignant tumours, 7th ed. ed. Wiley-Blackwell, Chichester, West Sussex, UK ; Hoboken, NJ.

Solé, X., Guinó, E., Valls, J., Iniesta, R., Moreno, V., 2006. SNPStats: a web tool for the analysis of association studies. Bioinformatics 22, 1928–1929. https://doi.org/10.1093/bioinformatics/btl268

Son, J., Lee, J.-J., Lee, J.-S., Schüller, A., Chang, Y.-T., 2010. Isozyme-specific fluorescent inhibitor of glutathione s-transferase omega 1. ACS Chem. Biol. 5, 449–453. https://doi.org/10.1021/cb100007s

Sosa, V., Moliné, T., Somoza, R., Paciucci, R., Kondoh, H., LLeonart, M.E., 2013. Oxidative stress and cancer: An overview. Ageing Research Reviews, Special Issue: Invertebrate Models of Aging 12, 376–390. https://doi.org/10.1016/j.arr.2012.10.004

Srigley, J.R., Delahunt, B., Eble, J.N., Egevad, L., Epstein, J.I., Grignon, D., Hes, O., Moch, H., Montironi, R., Tickoo, S.K., Zhou, M., Argani, P., ISUP Renal Tumor Panel, 2013. The International Society of Urological Pathology (ISUP) Vancouver Classification of Renal Neoplasia. Am. J. Surg. Pathol. 37, 1469–1489. https://doi.org/10.1097/PAS.0b013e318299f2d1

Stamenkovic, M., Radic, T., Stefanovic, I., Coric, V., Sencanic, I., Pljesa-Ercegovac, M., Matic, M., Jaksic, V., Simic, T., Savic-Radojevic, A., 2014. Glutathione S-transferase omega-2 polymorphism Asn142Asp modifies the risk of age-related cataract in smokers and subjects exposed to ultraviolet irradiation. Clin. Experiment. Ophthalmol. 42, 277–283. https://doi.org/10.1111/ceo.12180

Sweeney, C., Farrow, D.C., Schwartz, S.M., Eaton, D.L., Checkoway, H., Vaughan, T.L., 2000. Glutathione S-transferase M1, T1, and P1 polymorphisms as risk factors for renal cell carcinoma: a case-control study. Cancer Epidemiol. Biomarkers Prev. 9, 449–454.

Tanaka-Kagawa, T., Jinno, H., Hasegawa, T., Makino, Y., Seko, Y., Hanioka, N., Ando, M., 2003. Functional characterization of two variant human GSTO 1-1s (Ala140Asp and Thr217Asn). Biochemical and Biophysical Research Communications 301, 516–520. https://doi.org/10.1016/S0006-291X(02)03066-8

Teppo, H.-R., Soini, Y., Karihtala, P., 2017. Reactive Oxygen Species-Mediated Mechanisms of Action of Targeted Cancer Therapy. Oxid Med Cell Longev 2017, 1485283. https://doi.org/10.1155/2017/1485283

Tew, K.D., Townsend, D.M., 2012. Glutathione-S-Transferases As Determinants of Cell Survival and Death. Antioxid Redox Signal 17, 1728–1737. https://doi.org/10.1089/ars.2012.4640

The Cancer Genome Atlas Research Network, 2016. Comprehensive Molecular Characterization of Papillary Renal-Cell Carcinoma. N Engl J Med 374, 135–145. https://doi.org/10.1056/NEJMoa1505917

The International HapMap Consortium, 2005. A haplotype map of the human genome. Nature 437, 1299–1320. https://doi.org/10.1038/nature04226

Thijs, A.M.J., van Herpen, C.M.L., Verweij, V., Pertijs, J., van den Broek, P.H.H., van der Graaf, W.T.A., Rongen, G.A., 2015. Impaired endothelium-dependent vasodilation does not initiate the development of sunitinib-associated hypertension. J. Hypertens. 33, 2075–2082. https://doi.org/10.1097/HJH.0000000000000662

Tonelli, C., Chio, I.I.C., Tuveson, D.A., 2018. Transcriptional Regulation by Nrf2. Antioxidants & Redox Signaling 29, 1727–1745. https://doi.org/10.1089/ars.2017.7342

Towbin, H., Staehelin, T., Gordon, J., 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. U.S.A. 76, 4350–4354.

Tsuboi, K., Bachovchin, D.A., Speers, A.E., Brown, S.J., Spicer, T., Fernandez-Vega, V., Ferguson, J., Cravatt, B.F., Hodder, P., Rosen, H., 2010. Optimization and Characterization of an Inhibitor for Glutathione S-Tranferase Omega 1 (GSTO1), in: Probe Reports from the NIH Molecular Libraries Program. National Center for Biotechnology Information (US), Bethesda (MD).

Tsuboi, K., Bachovchin, D.A., Speers, A.E., Spicer, T.P., Fernandez-Vega, V., Hodder, P., Rosen, H., Cravatt, B.F., 2011. Potent and Selective Inhibitors of Glutathione S-transferase Omega 1 that Impair Cancer Drug Resistance. J Am Chem Soc 133, 16605–16616. https://doi.org/10.1021/ja2066972

Urzúa, U., Roby, K.F., Gangi, L.M., Cherry, J.M., Powell, J.I., Munroe, D.J., 2006. Transcriptomic analysis of an in vitro murine model of ovarian carcinoma: Functional similarity to the human disease and identification of prospective tumoral markers and targets. J. Cell. Physiol. 206, 594–602. https://doi.org/10.1002/jcp.20522

Valavanidis, A., Vlachogianni, T., Fiotakis, C., 2009a. 8-hydroxy-2′ -deoxyguanosine (8-OHdG): A Critical Biomarker of Oxidative Stress and Carcinogenesis. Journal of Environmental Science and Health, Part C 27, 120–139. https://doi.org/10.1080/10590500902885684

Valavanidis, A., Vlachogianni, T., Fiotakis, K., 2009b. Tobacco Smoke: Involvement of Reactive Oxygen Species and Stable Free Radicals in Mechanisms of Oxidative Damage, Carcinogenesis and Synergistic Effects with Other Respirable Particles. Int J Environ Res Public Health 6, 445–462. https://doi.org/10.3390/ijerph6020445

Wang, Y.-H., Yeh, S.-D., Shen, K.-H., Shen, C.-H., Juang, G.-D., Hsu, L.-I., Chiou, H.-Y., Chen, C.-J., 2009. A significantly joint effect between arsenic and occupational exposures and risk genotypes/diplotypes of CYP2E1, GSTO1 and GSTO2 on risk of urothelial carcinoma. Toxicology and Applied Pharmacology 241, 111–118. https://doi.org/10.1016/j.taap.2009.08.008

Wei, E.Y., Hsieh, J.J., 2015. A river model to map convergent cancer evolution and guide therapy in RCC. Nature Reviews Urology 12, 706–712. https://doi.org/10.1038/nrurol.2015.260

Weikert, S., Boeing, H., Pischon, T., Weikert, C., Olsen, A., Tjonneland, A., Overvad, K., Becker, N., Linseisen, J., Trichopoulou, A., Mountokalakis, T., Trichopoulos, D., Sieri, S., Palli, D., Vineis, P., Panico, S., Peeters, P.H.M., Bueno-de-Mesquita, H.B., Verschuren, W.M.M., Ljungberg, B., Hallmans, G., Berglund, G., Gonzalez, C.A., Dorronsoro, M., Barricarte, A., Tormo, M.J., Allen, N., Roddam, A., Bingham, S., Khaw, K.-T., Rinaldi, S., Ferrari, P., Norat, T., Riboli, E., 2008. Blood Pressure and Risk of Renal Cell Carcinoma in the European Prospective Investigation into Cancer and Nutrition. American Journal of Epidemiology 167, 438–446. https://doi.org/10.1093/aje/kwm321

Westervelt, P., 2001. Sudden death among patients with acute promyelocytic leukemia treated with arsenic trioxide. Blood 98, 266–271. https://doi.org/10.1182/blood.V98.2.266

Wettersten, H.I., Aboud, O.A., Lara, P.N., Weiss, R.H., 2017. Metabolic reprogramming in clear cell renal cell carcinoma. Nat Rev Nephrol 13, 410–419. https://doi.org/10.1038/nrneph.2017.59

Wettersten, H.I., Hakimi, A.A., Morin, D., Bianchi, C., Johnstone, M.E., Donohoe, D.R., Trott, J.F., Aboud, O.A., Stirdivant, S., Neri, B., Wolfert, R., Stewart, B., Perego, R., Hsieh, J.J., Weiss, R.H., 2015. Grade-Dependent Metabolic Reprogramming in Kidney Cancer Revealed by Combined Proteomics and Metabolomics Analysis. Cancer Res. 75, 2541–2552. https://doi.org/10.1158/0008-5472.CAN-14-1703

Whitbread, A.K., Masoumi, A., Tetlow, N., Schmuck, E., Coggan, M., Board, P.G., 2005. Characterization of the Omega Class of Glutathione Transferases, in: Methods in Enzymology. Elsevier, pp. 78–99. https://doi.org/10.1016/S0076-6879(05)01005-0

Whitbread, A.K., Tetlow, N., Eyre, H.J., Sutherland, G.R., Board, P.G., 2003. Characterization of the human Omega class glutathione transferase genes and associated polymorphisms. Pharmacogenetics 13, 131–144. https://doi.org/10.1097/01.fpc.0000054062.98065.6e

Wilk, J.B., Walter, R.E., Laramie, J.M., Gottlieb, D.J., O'Connor, G.T., 2007. Framingham Heart Study genome-wide association: results for pulmonary function measures. BMC Med Genet 8, S8. https://doi.org/10.1186/1471-2350-8-S1-S8

Wu, B., Dong, D., 2012. Human cytosolic glutathione transferases: structure, function, and drug discovery. Trends in Pharmacological Sciences 33, 656–668. https://doi.org/10.1016/j.tips.2012.09.007

Wu, F., Wu, S., Tong, H., He, W., Gou, X., 2019. HOXA6 inhibits cell proliferation and induces apoptosis by suppressing the PI3K/Akt signaling pathway in clear cell renal cell carcinoma. Int J Oncol. https://doi.org/10.3892/ijo.2019.4789

Xie, Y., Dahlin, J.L., Oakley, A.J., Casarotto, M.G., Board, P.G., Baell, J.B., 2018. Reviewing Hit Discovery Literature for Difficult Targets: Glutathione Transferase Omega-1 as an Example. J. Med. Chem. 61, 7448–7470. https://doi.org/10.1021/acs.jmedchem.8b00318

Xu, Y.-T., Wang, Jun, Yin, R., Qiu, M.-T., Xu, Lei, Wang, Jie, Xu, Lin, 2014. Genetic polymorphisms in Glutathione S-transferase Omega (GSTO) and cancer risk: a meta-analysis of 20 studies. Sci Rep 4. https://doi.org/10.1038/srep06578

Yan, X., Pan, L., Yuan, Y., Lang, J., Mao, N., 2007. Identification of Platinum-Resistance Associated Proteins through Proteomic Analysis of Human Ovarian Cancer Cells and Their Platinum-Resistant Sublines. Journal of Proteome Research 6, 772–780. https://doi.org/10.1021/pr060402r

Yanbaeva, D.G., Wouters, E.F.M., Dentener, M.A., Spruit, M.A., Reynaert, N.L., 2009. Association of glutathione-S-transferase omega haplotypes with susceptibility to chronic obstructive pulmonary disease. Free Radical Research 43, 738–743. https://doi.org/10.1080/10715760903038440

Yang, X., Long, S., Deng, J., Deng, T., Gong, Z., Hao, P., 2013. Glutathione S-Transferase Polymorphisms (GSTM1, GSTT1 and GSTP1) and Their Susceptibility to Renal Cell Carcinoma: An Evidence-Based Meta-Analysis. PLoS ONE 8, e63827. https://doi.org/10.1371/journal.pone.0063827

Yin, Z.-L., Dahlstrom, J.E., Le Couteur, D.G., Board, P.G., 2001. Immunohistochemistry of Omega Class Glutathione S-Transferase in Human Tissues. J Histochem Cytochem. 49, 983–987. https://doi.org/10.1177/002215540104900806

Yoshida, N., Ikemoto, S., Narita, K., Sugimura, K., Wada, S., Yasumoto, R., Kishimoto, T., Nakatani, T., 2002. Interleukin-6, tumour necrosis factor α and interleukin-1β in patients with renal cell carcinoma. Br J Cancer 86, 1396–1400. https://doi.org/10.1038/sj.bjc.6600257

Yuan, J.M., Castelao, J.E., Gago-Dominguez, M., Ross, R.K., Yu, M.C., 1998. Hypertension, obesity and their medications in relation to renal cell carcinoma. Br. J. Cancer 77, 1508–1513. https://doi.org/10.1038/bjc.1998.248

Yun, J., Mullarky, E., Lu, C., Bosch, K.N., Kavalier, A., Rivera, K., Roper, J., Chio, I.I.C., Giannopoulou, E.G., Rago, C., Muley, A., Asara, J.M., Paik, J., Elemento, O., Chen, Z., Pappin, D.J., Dow, L.E., Papadopoulos, N., Gross, S.S., Cantley, L.C., 2015. Vitamin C selectively kills KRAS and BRAF mutant colorectal cancer cells by targeting GAPDH. Science 350, 1391–1396. https://doi.org/10.1126/science.aaa5004

Zakharyan, R.A., Aposhian, H.V., 1999. Enzymatic reduction of arsenic compounds in mammalian systems: the rate-limiting enzyme of rabbit liver arsenic biotransformation is MMA(V) reductase. Chem. Res. Toxicol. 12, 1278–1283.

Zakharyan, R.A., Sampayo-Reyes, A., Healy, S.M., Tsaprailis, G., Board, P.G., Liebler, D.C., Aposhian, H.V., 2001. Human monomethylarsonic acid (MMA(V)) reductase is a member of the glutathione-S-transferase superfamily. Chem. Res. Toxicol. 14, 1051–1057.

Zhang, J., Ye, Z., Singh, S., Townsend, D.M., Tew, K.D., 2018. An evolving understanding of the S-glutathionylation cycle in pathways of redox regulation. Free Radical Biology and Medicine 120, 204–216. https://doi.org/10.1016/j.freeradbiomed.2018.03.038

Zhong, Z., Li, H., Zhong, H., Zhou, T., Xie, W., Lin, Z., 2018. A systematic review and meta-analyses of the relationship between glutathione S-transferase gene polymorphisms and renal cell carcinoma susceptibility. BMC Med Genet 19. https://doi.org/10.1186/s12881-018-0620-y

Zhou, H., Brock, J., Liu, D., Board, P.G., Oakley, A.J., 2012. Structural Insights into the Dehydroascorbate Reductase Activity of Human Omega-Class Glutathione Transferases. Journal of Molecular Biology 420, 190–203. https://doi.org/10.1016/j.jmb.2012.04.014

Zhou, N., Lu, F., Liu, C., Xu, K., Huang, J., Yu, D., Bi, L., 2016. IL-8 induces the epithelial-mesenchymal transition of renal cell carcinoma cells through the activation of AKT signaling. Oncology Letters 12, 1915–1920. https://doi.org/10.3892/ol.2016.4900

Znaor, A., Lortet-Tieulent, J., Laversanne, M., Jemal, A., Bray, F., 2015. International Variations and Trends in Renal Cell Carcinoma Incidence and Mortality. European Urology 67, 519–530. https://doi.org/10.1016/j.eururo.2014.10.002

LIST OF ABREVIATIONS

8-OHdG: 8-hydroxy-2′-deoxyguanosine

Akt: protein kinase B

APS: amonium per-sulphate

BAP1: BRCA1 associated protein 1

BCA: bicinchoninic acid

BMI: bodi mass index

BSA: bovine serum albumine

CAFs: cancer associated fibroblasts

CCL2: chemokine (C-C motif) ligand 2

CCL5: chemokine (C-C motif) ligand 5

CCND1: cyclin D1 gene

ccRCC: clear renal cell carcinoma

CEBP β: CCAAT enhancer binding protein β

chRCC: chromophobe renal cell carcinoma

CI: confidence interval

CMFDA: 5-chloromethylfluoresceindiacetate

COPD: chronic obstructive pulmonary disease

CRID: cytokine release inhibitory drug

CT: computerized tomography

DHAR: dehydroascorbate-reductase

DMA^V: $dimethylarsenate^V$

DNA: deoxyribonucleic acid

DTT: dithiothreitol

ELISA: enzyme linked immunosorbent assays

EPAS1: gene encoding for hypoxia-inducible factor 2 alpha subunit

ERK: extracellular signal-regulated kinase

EZH2: enhancer of zeste homolog 2

FH: gene encoding for fumarase

FLCN: gene encoding for folliculin

FNR: Fuhrman nuclear grade

GLUT1: glucose transporter 1

GSH: glutathione

GST: glutathione transferase

GSTA1: glutathione transferase alpha 1

GSTM1: glutathione transferase mu 1

GSTO1: glutathione transferase omega 1

GSTO2: glutathione transferase omega 2

GSTP1: glutathione transferase pi 1

GSTT1: glutathione transferase theta 1

GWAS: Genome-Wide Association Studies

HIF: hypoxia-inducible factor

HR: hazard ratio

HRE: hypoxia-response element

HRP: horse radish peroxidase

IL-10: interleukin-10

IL-1β: interleukin-1β

IL-6: interleukin-6

IL-8: interleukin-8

IRAC: International Agency for Research on Cancer

ISUP: International Society of Urological Pathology

IκB-α: nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor α

Jak: Janus kinase

JNK1: c-Jun NH2-terminal kinase

Keap 1: Kelch-like ECH-associated protein 1

MAPK: mitogen activated kinase

MEK: mitogen-activated protein kinase kinase

miRNA: micro ribonucleic acid

MMA^{V}: $monomethylarsenate^{V}$

MMP: matrix metalloproteinase

MRI: magnetic resonance imaging

mRNA: messenger ribonucleic acid

mTOR: mammalian target of rapamycin

mTORC: mammalian target of rapamycin complex

nccRCC: non-clear cell renal cell carcinoma

NF-κB: nuclear factor kappa B

Nrf2: nuclear factor erythroid 2-related factor 2

OR: odds ratio

PBRM1: polybromo1

PCR: polymerase chain reaction

PDGFR beta: platelet-derived growth factor receptor-beta

PDK1: phosphoinositide-dependent kinase-1

PI3K: phosphoinositide 3-kinase

pRCC: papillary renal cell carcinoma

PTEN: phosphatase and tensin homologue deleted on chromosome 10

PYK2: proline-rich tyrosine kinase 2

RCC: renal cell carcinoma

RFLP: restriction fragment length polymorphism

ROS: reactive oxygen species

RPS6: ribosomal protein S6

RSK1p90: 90 kDa ribosomal protein S6 kinase 1

RyR: ryanodine receptor

SDHB: gene encoding for succinate dehydrogenase iron-sulfur subunit

SDHC: succinate dehydrogenase complex subunit C

SDHD: succinate dehydrogenase complex subunit D

SDS-PAGE: sodium dodecyl sulfate-polyacrilamide gel electrophoresis

SETD2: SET domain containing 2

SNP: single nucleotide polyorphism

SQSTM1: sequestosome-1

SRC: proto-oncogene tyrosine-protein kinase Src

STAT: signal transducer and activator of transcription

STC2: stanniocalcin-2

TCA: citric acid cycle

TCC: transitional cell carcinoma

TGFα: transforming growth factor α

TNF-α: tumor necrosis factor α

TNM: tumor–node–metastasis

TSC1: gene encoding for hamartin

TSC2: gene encoding for tuberin

UICC: Union for International Cancer Control

VCAN: gene encoding for versican

VEGF: vascular endothelial growth factor

VEGF-R: vascular endothelial growth factor receptor

VHL: von Hippel Lindau

WHO: World health organisation

BIOGRAPHY

Tanja Radić (maiden name Jevtić) was born in Šabac in 1985, where she completed her elementary and high school education with honors. She graduated from the Faculty of Biology, University of Belgrade with master's degree in Molecular biology and physiology in 2012, with average grade 8.97/10.00.

In 2012 Tanja Radić joined the research group headed by Professor dr Tatjana Simić at the Institute of Medical and Clinical Biochemistry, Faculty of Medicine, University of Belgrade as a Research Assistant, on project No. 175052 entitled "The role of glutathione S-transferase polymorphisms in susceptibility to disease development", financed by the Serbian Ministry of Education and Science. In 2012/2013 she enrolled in her PhD studies at the Faculty of Medicine, University of Belgrade. She participated in numerous domestic and foreign conferences. Tanja Radić attended the „Performing and analysis of high resolution array-based comparative genomic hybridization experiments on Clinical Human Samples" training in Waldbronn (Germany) in 2014, „School of Proteomics - Theoretical and Practical Basis" in Belgrade (Serbia) in 2015, and „FEBS Advanced Lecture Course 2018: Redox-omic Technologies and their Application in Health and Disease" summer school in Spetses (Greece). She was awarded with the travel grants for the participation in „FEBS Advanced Lecture Course 2018" (Spetses, Greece) and SFRR-E Meeting 2019: "Redox Homeostasis: from Signaling to Damage" Ferrara (Italy).

Tanja Radić is the author/co-author of 15 in extenso papers published in journals indexed in the Science Citation Index (SCI). Her papers have been cited 82 times according to SCOPUS, and her h index is 5.

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