Renal Cell Carcinoma Fuhrman Grade and Histological Subtype Correlate With Complete Polymorphic Deletion of Glutathione S-Transferase M1 Gene

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Abbreviations and Acronyms

ASK1 = apoptosis signal-regulating kinase 1

GST = glutathione S-transferase

PCR = polymerase chain reaction

RCC = renal cell carcinoma

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Purpose: We outlined the putative significance of GST in renal cell carcinoma biology by investigating the influence of its deletion polymorphisms on renal cell carcinoma progression.

Materials and Methods: Genomic DNA was purified from peripheral blood leukocytes. GSTM1 and GSTT1 genes were polymerase chain reaction amplified and gene fragments were separated by agarose gel electrophoresis. Intact GSTM1 and GSTT1 alleles were identified by the presence of 230 and 480 bp fragments, respectively. Genotypes were associated with clinicopathological variables and survival.

Results: Of 147 patients with renal cell carcinoma 80 (54%) had the GSTM1 null and 27 (18%) had the GSTT1 null genotype. The GST genotype distribution did not differ significantly from that in 112 controls without renal cell carcinoma. However, the GSTM1 null genotype was associated with 60% lower odds of the papillary subtype (OR 0.40, 95% CI 0.18 to 0.92, p = 0.032), lower Fuhrman grade (chi-square 9.77, p = 0.008) and a lower risk of metastatic disease in patients with the clear cell subtype (chi-square 4.48, p = 0.034). Of patients with the clear cell subtype those with the GSTM1 null genotype had improved cancer specific survival (p = 0.0412). GSTT1 did not correlate with any pathological variable except age at renal cell carcinoma onset since patients with renal cell carcinoma and the GSTT1 null genotype were significantly younger than their counterparts (mean ± SD age 58.5 ± 14.2 vs 65.4 ± 12.8 years, p = 0.016).

Conclusions: GSTM1 deletion polymorphism impacts renal cell carcinoma histological subtype, Fuhrman grade and metastatic behavior while GSTT1 deletion leads to renal cell carcinoma onset at a younger age. In patients with clear cell renal cell carcinoma the GSTM1 null genotype may be associated with better prognosis.

Key Words: kidney; carcinoma, renal cell; glutathione transferase; polymorphism, genetic; gene deletion

In past decades our understanding of RCC biology has improved significantly. Genetic syndromes leading to RCC have been described, histological subtypes with distinct biological pathways have been delineated and novel drugs have been developed.[1] However, most cases are sporadic, their actual etiology is unknown and RCC progression remains poorly understood.[1,2] Recent studies indicate that mutations and aberrations in certain genes, such as deletions in GST genes, may impact RCC pathogenesis.[3–5]

Cytosolic GSTs are subdivided into 7 classes, including α, μ, ω, π, σ,

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θ and ζ.[6] GSTs are generally considered detoxification enzymes that catalyze the conjugation of glutathione with a wide variety of xenobiotics, including carcinogens and chemotherapeutic agents, thereby protecting cells from environmental and oxidative stress.[6] In addition to their detoxifying role in the kidney, metabolism of certain substrates through GSTs leads to more reactive products that damage the kidney,[4] which in turn leads to the progression of renal diseases such as RCC. Recent studies show that GSTs regulate cell survival, proliferation and apoptosis by acting as ligand binding proteins for cell cycle components such as mitogen activated protein kinases.[7]

GSTM1, the gene encoding for the GST-μ1 enzyme, is located on chromosome 1p13.3. Complete deletions of this gene (GSTM1 null genotype) have been detected in about 50% of the white population.[8] Individuals with this deletion polymorphism do not synthesize GST-μ1 and its detoxifying function is completely lost.[6] As a consequence, the GSTM1 null genotype is associated with an increased risk of certain cancer types, such as ovarian and bladder cancer.[9–12] The GSTM1 null genotype is associated with a risk of RCC when it is present together with other certain genotypes.[8,10] Karami et al recently noted a correlation between GSTM1 and RCC risk in individuals exposed to pesticide.[4]

The GSTT1 gene, located on chromosome 22q11.23, encodes for the enzyme GST-θ1. Polymorphic deletions of the GSTT1 gene (null genotype) have been detected in 15% to 20% of the white population and are associated with decreased GST-θ1 catalytic activity.[6,10] Studies show an increased risk of cancer of the head, neck and oral cavity in individuals with the GSTT1 null genotype.[13] In several studies the GSTM1 and GSTT1 genotypes were associated with survival and response in patients undergoing treatment for ovarian cancer and acute myeloid leukemia.[14–16] Thus, there is also mounting evidence that GST participates in carcinoma progression.

Intrigued by these observations, we elucidated the putative role of GSTM1 and GSTT1 in RCC biology by investigating the influence of their deletions on RCC progression.

PATIENTS AND METHODS

Patient Population

A total of 259 Austrian individuals were enrolled in this hospital based study, including 147 consecutive patients treated for sporadic RCC at the University of Vienna department of urology between 2004 and 2009, and 112 controls with no evidence or history of cancer. Written informed consent was obtained from all patients and the study was approved by the local ethics committee.

DNA Purification and PCR

Genomic DNA was purified from 10 ml ethylenediaminetetraacetic acid supplemented peripheral blood using the Wizard® Genomic DNA Purification Kit. Tumor DNA was not used in any case. GSTT1 and GSTM1 genotypes were determined by multiplex PCR. We used β-globulin as a positive control for successful amplification to ensure that a missing band represented GST allele deletion and not amplification failure. The primer pairs used for PCR amplification were 5′–GAA CTC CCT GAA AAG CTA AAG C–3′ 5′–GTT GGG CTC AAA TAT ACG GTG G–3′ for GSTM1, 5′–TTC CTT ACT GGT CCT CAC ATC TC–3′ 5′–TCA CCG GAT CAT GGC CAC CA–3′ for GSTT1 and 5′–ACA CAA CTG TGT TCA CTA GC–3′ 5′–CAA CTT CAT CCA CGT TCA CC–3′ for β-globulin.

PCR products were obtained in a 25 μl mixture consisting of 1 × PCR buffer, 1.5 mM $MgCl_2$, 100 ng DNA, 0.2 mM nucleotide solution, 30 pmol of primers and 0.3 U Platinum® Taq DNA Polymerase. PCR reactions were done for 35 cycles, each including a 30-second denaturation step at 94C, a 30-second annealing step at 62C and a 40-second extension step at 72C. The PCR program included an initial denaturation time of 10 minutes at 94C and an extension time of 7 minutes at 72C after the last cycle. PCR products were separated by electrophoresis using 1.5% agarose gel and visualized by ethidium bromide staining.

GST Genotype Determination

The presence of 230 and 480 bp bands corresponded to intact genomic GSTM1 and GSTT1, respectively, while the absence of the bands implied a null genotype (simultaneous deletion of each allele) (fig. 1). GSTM1 and GSTT1 variations were evaluated as active when participants had at least 1 nondeleted GSTM1 or GSTT1 allele and inactive when the 2 copies of each gene were deleted.[4]

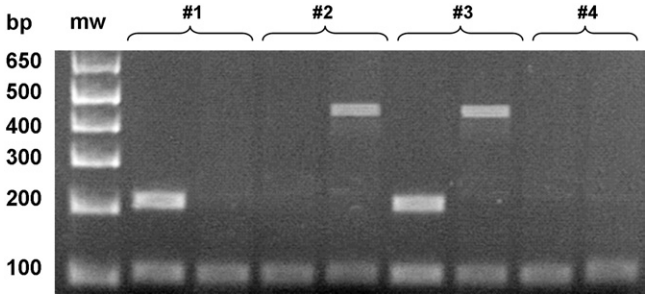

Figure 1. Multiplex PCR shows GSTT1 and GSTM1 genotypes. Amplified gene fragments were separated on 1.5% agarose gels and visualized by ethidium bromide staining. GSTM1 or GSTT1 alleles were identified by 230 and 480 bp bands, respectively, and β-globulin at 110 bp served as positive control. *mw*, molecular weight. *#1*, patient 1 GSTM1 active and GSTT1 inactive. *#2*, patient 2 GSTM1 inactive and GSTT1 active. *#3*, patient 3 GSTM1 active and GSTT1 active. *#4*, patient 4 GSTM1 inactive and GSTT1 inactive.