JBUON 2018; 23(6): 1887-1892
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
E-mail: editorial_office@jbuon.com

ORIGINAL ARTICLE

HTERT promoter methylation and single nucleotide polymorphism (-245 T>C) affect renal cell carcinoma behavior in Serbian population

Jovanka Trifunovic[1], Gordana Basta-Jovanovic[1], Nadja Nikolic[2], Jelena Carkic[2], Ana Marjanovic[3], Marija Brankovic[3], Sanja Radojevic-Skodric[1], Mirjana Prvanovic[1], Aleksandar Jovanovic[4], Zoran Dzamic[4], Jelena Milasin[2]

[1]Institute of Pathology, School of Medicine, University of Belgrade, Belgrade, Serbia; [2]Institute of Human Genetics, School of Dental Medicine, University of Belgrade, Belgrade, Serbia; [3]School of Medicine, University of Belgrade, Belgrade, Serbia; [4]Clinic of Urology, Clinical Center of Serbia, School of Medicine, University of Belgrade, Belgrade, Serbia

Summary

Purpose: *Renal cell carcinoma (RCC) is the most common renal cancer in adults and includes several subtypes that may be distinguished by their histology, genetic background, clinical course and responses to treatment. Human telomerase reverse transcriptase (hTERT), a crucial enzyme for telomere maintenance, has been linked to RCC development. The purpose of this study was to search for genetic and epigenetic alterations in hTERT (promoter mutations and methylation and gene amplification), and to establish a possible association between molecular and clinico-pathological characteristics of RCC.*

Methods: *DNA was extracted from 31 formalin-fixed, paraffin-embedded tumor samples and 23 blood samples from 54 patients with RCC. Polymerase chain reaction (PCR) products were sequenced and analyzed using the Sequencher software. HTERT amplification was determined by quantitative PCR, while the promoter methylation status was assessed by methylation specific PCR. Statistical analysis was performed using SPSS.*

Results: *No mutations could be detected in the hTERT promoter but only a single nucleotide polymorphism (SNP) (-245 T>C). In 54 analyzed RCC cases, the variant allele C was present in homozygous or heterozygous form in 48% of the patients. The C allele was significantly more frequent in low grade tumors (p=0.046). Gene amplification was detected in 19.4% of the 31 RCCs and hTERT methylation in 54.8% of the 31 samples. An association was established between methylation and histological type of RCC (p=0.047).*

Conclusions: *HTERT seems to be implicated in RCC pathogenesis since the promoter polymorphism exerts a modulation effect on tumor behavior. In addition, hTERT promoter methylation status is related to RCC histology.*

Key words: *gene amplification, hTERT, methylation, polymorphism, promoter, RCC*

Introduction

Renal cell carcinoma (RCC) is the prevailing form of renal cancer in adults, accounting for approximately 3% of all cancer diagnoses [1-4]. RCC includes several subtypes that may be distinguished by their histology, natural history and responses to therapy [4]. Clear cell carcinoma (ccRCC) is the most frequent tumor subtype, comprising around 80% of the RCCs [5].

Due to its resistance to chemotherapy and radiotherapy RCC is a very aggressive and often fatal

Correspondence to: Dr Jelena Milasin. Dr Subotica 8, 11000 Belgrade, Serbia
Tel: + 381 11 2685288, Fax: +381 11 2685361, E-mail: jelena.milasin@stomf.bg.ac.rs
Received: 19/02/2018; Accepted: 15/03/2018

disease. Partial or complete response to immunobiologic therapy is noticed only in a small number of patients, mainly due to the absence of specific tumor antigens [6,7]. The typical onset of RCC is between 50-70 years of age with male predominance [3,4,8]. Despite improvements in diagnostic and therapeutic approaches, RCC incidence and mortality are still rising in developing countries, while overall and disease free survival rates vary considerably between lower-income and developed countries [2,9-11].

Though large-scale genetic and epigenetic studies of RCC pathogenesis have brought important knowledge into the molecular mechanisms of RCC pathogenesis, it still remains insufficiently understood. Mechanisms involving telomerase have also been linked to risk and progression of RCC [12,13]. Telomerase is a ribonucleoprotein complex required for chromosomal stability. It consists of human telomerase reverse transcriptase (hTERT), a catalytic protein which replicates the ends of linear DNA molecules called telomeres, and intrinsic human telomerase RNA (hTR) which serves as base template for replication [14,15]. HTERT prevents the harmful consequences of exposed DNA ends, such as chromosomal end-to-end fusions and nucleolytic processing [16].

Telomerase is expressed in germ cells, actively proliferating cells of renewing tissues, and immortalized cells [17]. The progressive reduction of telomerase expression in postnatal somatic cells with consequent telomere shortening with every mitotic division is related to cellular senescence [18]. Activation of telomerase and stabilization of telomeres are consistent with the hypothesis that telomere maintenance is essential for cancer cells to achieve immortality [19].

Telomerase activity of variable level has been detected in approximately 80-90% of human cancer specimens [20-22]. HTERT expression may be affected by different genetic and epigenetic mechanisms, e.g. mutations, gene rearrangements, the presence of SNPs, methylation, etc.

The purpose of the present study was to assess: (a) the presence of mutations and −245T > C SNP variant in the promoter of hTERT; (b) the presence of hTERT amplification; (c) the promoter methylation status, and (d) to search for a possible association between molecular and clinical/pathological characteristics of renal cell carcinomas in Serbian population.

Methods

Samples

This retrospective study included 31 formalin-fixed, paraffin-embedded (FFPE) samples and 23 blood samples, originating from 54 patients with RCC (surgically treated at the Clinic of Urology, Clinical Centre of Serbia and the Clinical Hospital Center "Dragisa Misovic-Dedinje", diagnosed at the Institute of Pathology, School of Medicine, University of Belgrade). The study protocol was approved by the Ethics Committee of both institutes. The study was performed in accordance with the Declaration of Helsinki. Of the 54 patients, 31 (57.4%) were male and 23 (42.6%) female; the median age at the time of surgical treatment was 59 years (range 33-85) (Table 1). On the basis of the median size of the tumors, they were classified as small-sized (<50mm) and large-sized (≥50mm). Thirty cases (55.5%) were classified as grade I/II (low grade) and 24 (44.5%) as grade III/IV (high grade). Stages T1 and T2 were classified as low-stage tumors (28 out of 54 cases; 51.9%). T3 and T4 were high-stage tumors (26 cases; 48.1%). Three different histological types of RCC were present: clear cell (ccRCC) - 36 cases (66.7%), papillary (pRCC) - 13 patients (24.1%) and chromophobe (chRCC) - 5 patients (9.2%). Median survival time was 38 months, ranging from 11 to 54 months.

DNA extraction

Genomic DNA was extracted from FFPE samples after de-paraffinisation, using standard phenol-chloroform extraction protocol, and from blood using high salt extraction method.

Table 1. Distribution of hTERT SNP genotypes, hTERT gene amplification and promoter hypermethylation in relation to patients age and gender

	hTERT polymorphism			*hTERT amplification*			*hTERT methylation*		
	wt n (%)	*het/mut* n (%)	*p value*	*no* n (%)	*yes* n (%)	*p value*	*no* n (%)	*yes* n (%)	*p value*
Age (years)			0.571			0.634			0.119
<59	14 (56)	11 (48)		11 (84.6)	2 (15.4)		8 (61.5)	5 (38.5)	
≥59	14 (48.3)	15 (51.7)		14 (77.8)	4 (22.2)		6 (33.3)	12 (66.7)	
Gender			0.289			0.083			0.108
Male	18 (58.1)	13 (41.9)		14 (93.3)	1 (6.7)		9 (60)	6 (40)	
Female	10 (43.5)	13 (56.5)		11 (68.8)	5 (31.3)		5 (31.3)	11 (68.8)	

PCR amplification and sequencing

A fragment of 343 bp corresponding the region from -278 to +65 within hTERT gene was amplified with the following primers: forward 5'-AGCACCTCGCGGTAGTGG-3' and reverse 5'-GGATTCGCGGGCACAGAC-3'. The PCR amplifications were performed using PCR Master Mix (2X) (Thermo Fisher Scientific, Vilnius, Lithuania) in 20 µl reaction volume with 0.25 µM of each primer. The temperature conditions were: 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 55°C for 45 s, and extension at 72°C, for 1 min, and a final extension at 72°C for 5 min. The presence of PCR products was confirmed by electrophoresis on 8% PAA gels. The amplification products were directly sequenced in forward and reverse directions using the ABI Big Dye Terminator chemistry and an ABI 3500 instrument (Applied Biosystems, Foster City, CA, USA). The sequences were analyzed using the Sequencher software (Gene Codes Corporation, Ann Arbor, MI). Identified sequence changes were confirmed by analyzing each sample in duplicate.

Methylation specific PCR

The hTERT promoter methylation status was determined by methylation specific PCR (MSP). Genomic DNA was modified by sodium bisulphite treatment with EZ-DNA methylation kit, according to manufacturer's recommendations (Zymo Research, Orange, CA, USA). In order to detect DNA sequence alterations after bisulphite treatment, MSP was used to distinguish methylated from unmethylated alleles. Two separate PCR reactions were using the following primers: Uf: 5'-TTGAGAATTTGTAAAGAGAAATGATG-3' and Ur: 5'-CTAAAAACAAACCCAAAAAACACA-3' for unmethylated promoter (product length 133 bp) and Mf: 5'-TTGAGAATTTGTAAAGAGAAATGAC-3' and Mr: 5'-TAAAAACGAACCCGAAAACG-3' for methylated promoter (product length 131 bp). PCR amplification was performed in a total volume of 50 µl and the reaction mix contained 1 × PCR Buffer (10 mM Tris-HCl, 50 mM KCl, 0.1% Triton X-100), 8 mM $MgCl_2$, 1.25 mM dNTPs, 0.6 µM primers (Invitrogen, Life Technologies, Carlsbad, CA, USA), 0.4 µg/µl BSA, 5% DMSO, 1.5U *Taq* polymerase (Thermo Scientific, Hudson, NH, USA) and 3 µl of bisulfite treated DNA as a template. Amplification was under the following conditions: initial denaturation at 95°C for 5 min, followed by 40 cycles 95°C denaturation for 30 s, annealing for 30 s at 55°C, 72°C extensions for 30 s, and final extension at 72°C for 4 min. Genomic DNA from lymphocytes of healthy donors was used as control for unmethylated genes and the same DNA, treated *in vitro* with SssI methyltransferase, was used as control for methylated genes. PCR products were loaded on 8% PAA gels, stained with ethidium bromide and visualized under the UV light. HTERT gene promoter was considered as (1) non-methylated, when only the reaction with the unmethylated (U) primers showed amplification and (2) methylated/partially methylated, when amplification was present only with the methylated (M) primer pair or with both (U and M). Random re-amplification of 20% of samples was done and there were no discrepancies between methylation statuses determined in duplicate.

Quantitative Real time PCR (qPCR) analysis

A quantitative real-time polymerase chain reaction (qPCR) and comparative cycle threshold (Ct) method of quantitation of hTERT gene was performed using SensiFAST™ SYBR® Hi-ROX Kit (Bioline, London, UK), according to the manufacturer`s recommendation. In parallel, β-globin gene was amplified as a reference for normalization. All real-time PCR experiments were performed in duplicate. Primers used for the amplification were: 5'-CACCCGTCCTGCCCCTTCACCTT-3' and 5'-GGCTTCCCACGTGCGCAGCAGGA-3' for hTERT and 5'-TGTGCTGGCCCATCACTTTG-3' and 5'-ACCAGCCACCACTTTCTGATAGG-3' for β-globin. The Ct value was calculated for each sample and the amplification levels were calculated as $2^{-\Delta\Delta Ct}$. Amplification level of hTERT was normalized against β-globin and a gene dose greater than 2.5 was considered as amplification.

Statistics

Statistical analyses were performed using SPSS Statistics for Windows Software (Version 22.0, IBM Corp, Armonk, NY). Descriptive analysis of data included mean ± standard deviation and percentages. Pearson's chi-square test (x^2) and Mann-Whitney U-test were used to determine statistical significance between categorical and numerical data, respectively. All p values of 0.05 or less were considered as statistically significant.

Results

Mutations and SNPs screening

Sequencing of the 343 bp long promoter region spanning from -278 to +65 within the hTERT gene did not reveal activating mutations in any of the 31 RCC tumor specimens. Sequencing, however, detected the presence of a SNP at -245 bp position (rs2853669), a T to C transition. In order to perform an association study between SNP presence and clinical parameters, we increased the initial sample with 23 new DNAs obtained from blood of additional 23 RCC patients and determined their genotypes.

Out of 54 analyzed RCC cases, the variant allele was present in homozygous form in only 4 cases (7.4%) and in heterozygous in 22 cases (40.7%), while the remaining 28 cases (51.9%) were homozygous for the wild type allele. The probability of having a low grade RCC was 3 times higher in carriers of the variant allele than in wild type (TT) homozygotes (Table 2).

hTERT amplification

Gene amplification was detected in 6 (19.4%) of the 31 analyzed tumor samples. There was no association between hTERT amplification and epidemiological (Table 1), clinical and pathological findings (Table 2). The levels of amplification ranged between 2.60 and 7.61.

Table 2. Distribution of hTERT SNP genotypes, gene amplification and promoter hypermethylation in relation to clinical and pathological data

	hTERT polymorphism			*hTERT amplification*			*hTERT methylation*		
	wt n (%)	*het/mut* n (%)	*p value*	*no* n (%)	*yes* n (%)	*p value*	*no* n (%)	*yes* n (%)	*p value*
Histological type			0.282			0.441			0.047
pRCC	8 (61.5)	5 (38.5)		6 (85.7)	1 (14.3)		5 (71.4)	2 (28.6)	
ccRCC	19 (52.8)	17 (47.2)		16 (84.2)	3 (15.8)		9 (47.4)	10 (52.6)	
chRCC	1 (20)	4 (80)		3 (60)	2 (40)		0 (0)	5 (100)	
Stage			0.777			0.162			0.542
Low	14 (50)	14 (50)		13 (72.2)	5 (27.8)		9 (50)	9 (50)	
High	14 (53.8)	12 (46.2)		12 (92.3)	1 (7.7)		5 (38.5)	8 (61.5)	
Grade			0.046			0.656			0.925
Low	12 (40)	18 (60)		15 (83.3)	3 (16.7)		8 (44.4)	10 (55.6)	
High	16 (66.7)	8 (33.3)		10 (76.9)	3 (23.1)		6 (46.2)	7 (53.8)	
Tumor size, mm			0.419			0.791			0.092
<50	12 (46.2)	14 (53.8)		11 (78.6)	3 (21.4)		4 (28.6)	10 (71.4)	
≥50	16 (57.1)	12 (42.9)		14 (82.4)	3 (17.6)		10 (58.8)	7 (41.2)	
Survival, months			0.571			0.656			0.524
<38	14 (56)	11 (44)		10 (76.9)	3 (23.1)		5 (38.5)	8 (61.5)	
≥38	14 (48.3)	15 (51.7)		15 (83.3)	3 (16.7)		9 (50)	9 (50)	

hTERT methylation

HTERT promoter was methylated in 17 of the 31 tumor samples (54.8%). Interestingly, in 71% of papillary carcinomas the promoter was unmethylated, whereas in 100% of chromophobe carcinomas it was methylated (Table 2).

Discussion

RCC is included among the most lethal cancers of the urinary system. Given its biological and clinical heterogeneity, new markers that would better determine RCC behavior are still needed.

Telomeres and telomerase have an important role in the initiation and progression of malignancies [23,24]. A majority of cancers are found to upregulate the expression of telomerase, conferring cancer cells the capacity to proliferate indefinitely [25]. hTERT gene expression is regulated at various molecular levels, but the transcription level seems to be the most important. Several genetic and epigenetic factors, such as promoter mutations, gene amplification and rearrangements, the presence of SNPs, methylation, etc. affect transcription and may influence hTERT expression and function [26].

There are still many controversies regarding the role that hTERT plays in RCC development in general, and in particular regarding the frequency of somatic mutations in the promoter, the level of activity/expression of telomerase in different RCC subtypes, the impact of SNPs etc [27].

In the present study we sequenced the hTERT promoter region of 31 tumor samples of RCC patients and no activating mutations in the expected spots could be identified. A limited number of studies have dealt with hTERT mutations in RCC and relatively low incidence of mutations, ranging between 6 and 13% have been reported [5,27,28], which is in line with our findings. The low frequencies of hTERT mutations in RCC are in sharp contrast with the results for urothelial tumors in which up to 100% of cases harbored hTERT mutations [29,30].

We further analyzed the frequency of gene amplification and found that almost 20% of RCC patients harbored this genetic alteration. A similar frequency of TERT gene amplification was detected in acral lentiginous melanoma and was correlated with poor outcome [31]. High frequencies of hTERT amplification (79%) were previously described in Merkel cell carcinoma [32] and were also associated with poor prognosis. In our sample, hTERT amplification did not appear to be informative in terms of disease outcome prediction as no association between amplification and specific tumor behavior could be established.

HTERT promoter methylation has not been investigated previously in RCCs. Our study is the first to consider the possible association of this epigenetic phenomenon with RCC behavior. Interestingly, hTERT promoter methylation was a rather frequent event, pointing to the importance of this

mechanism of hTERT gene regulation in some RCC subtypes. In total, over one half of tumor DNA samples exhibited promoter methylation, which is quite similar to findings in urothelial carcinoma of the bladder [33]. However, methylation strongly depended on the histological type; methylation frequencies were 29, 53 and 100% in pRCC, ccRCC and chRCC respectively, and thus it seemed to be a hallmark of chRCC, as all the samples were methylated. This finding, however, must be taken with caution due to the small number of analyzed samples. Such a high frequency of promoter methylation (100%) is not common, but has previously been described in different tumors and different genes. For instance, in mucoepidermoid carcinomas of the salivary glands p14 gene was methylated in 100% of the cases [34]. HTERT was also found to be methylated in 100% of oral squamous cell carcinomas [35]. A recent comprehensive study of the genomic landscape of chRCC established that this rare type of RCC displayed more hypomethylation than hypermethylation events compared to ccRCC, but the authors did not mention hTERT. They also noted that differences in DNA methylation pattern between the two types were considerable [36].

Promoter sequencing of tumor DNA samples revealed a relatively high frequency of the polymorphism rs2853669. Thus, in order to increase the power of the association study, we decided to analyze additional DNAs originating from patients' blood, aiming to find a relationship between the presence of this SNP and tumors characteristics. Indeed, it appeared that the presence of the variant allele has a protective effect, that is, patients carrying at least one variant allele (either as heterozygotes or homozygotes) had mostly low grade tumors. A study on non-small cell lung cancer showed also that CC genotype had significantly longer median survival time [37]. A large meta-analysis has not confirmed the role of this SNP, neither as a risk nor a protective factor in human cancer, except in the case of TT genotypes accompanied with hTERT mutations, when this was a predictor of poor survival [38].

In conclusion, hTERT, via its promoter methylation and functional promoter polymorphism, seems to play a role in RCC biology in Serbian patients. A larger sample is needed in order to confirm these findings.

Acknowledgements

This work was financially supported by the grants No. 175075 and 175059 of the Ministry of Education, Science and Technological Development, Republic of Serbia.

Conflict of interests

The authors declare no conflict of interests.

References

1. Ferlay J, Shin H-R, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010;127:2893-917.
2. Medina-Rico M, López-Ramos H, Lobo M, Romo J, Prada JG. Epidemiology of renal cancer in developing countries: Review of the literature. Can Urol Assoc J 2017;12. (epub ahead of print).
3. Jemal A, Siegel R, Xu J, Ward E. Cancer Statistics, 2010. CA Cancer J Clin 2010;60:277-300.
4. Rini BI, Campbell SC, Escudier B. Renal cell carcinoma. Lancet 2009;373:1119-32.
5. Hosen I, Rachakonda PS, Heidenreich B et al. TERT promoter mutations in clear cell renal cell carcinoma. Int J Cancer 2015;136:2448-52.
6. Lam JS, Belldegrun AS, Figlin RA. Advances in immune-based therapies of renal cell carcinoma. Expert Rev Anticancer Ther 2004;4:1081-96.
7. Chin AI, Lam JS, Figlin RA, Belldegrun AS. Surveillance strategies for renal cell carcinoma patients following nephrectomy. Rev Urol 2006;8:1-7.
8. de Wit R, Sternberg CN. Cancers of the genitourinary tract. Textbook of Medical Oncology 2004;20: 213-34.
9. Tsimafeyeu I, Zolotareva T, Varlamov S et al. Five-year survival of patients with metastatic renal cell carcinoma in the Russian Federation: results from the RENSUR5 Registry. Clin Genitourin Cancer 2017;15:e1069-72.
10. Vartolomei MD, Matei DV, Renne G et al. Robot-assisted partial nephrectomy: 5-yr oncological outcomes at a single European tertiary cancer center. Eur Urol Focus 2017 Oct 27 (epub ahead of print).
11. Pavlovic I, Pejic S, Glumac S et al. Clinicopathological characteristics and survival in Serbian patients with renal cell carcinoma: a retrospective analysis. JBUON 2017;22:1434-40.
12. Mekhail TM, Kawanishi-Tabata R, Tubbs R et al. Renal cell carcinoma (RCC) and telomerase activity: relationship to stage. Urol Oncol 2003;21:424-30.
13. Fujioka T, Hasegawa M, Suzuki Y et al. Telomerase activity in human renal cell carcinoma. Int J Urol 2000;7:16-21.
14. Shay JW, Zou Y, Hiyama E, Wright WE. Telomerase and cancer. Hum Mol Genet 2001;10:677-85.
15. Feng J, Funk WD, Wang SS et al. The RNA component of human telomerase. Science 1995;269:1236-41.

16. de Lange T. How telomeres solve the end-protection problem. Science 2009;326:948-52.
17. Shay JW, Wright WE. Telomeres and telomerase in normal and cancer stem cells. FEBS Lett 2010;584:3819-25.
18. Hiyama E, Hiyama K. Telomere and telomerase in stem cells. Br J Cancer 2007;96:1020-24.
19. Tsanev R. Molecular mechanisms of cancer cells survival. JBUON 2005;10:309-18.
20. Holt SE, Shay JW. Role of telomerase in cellular proliferation and cancer. J Cell Physiol 1999;180:10-8.
21. Carkic J, Nikolic N, Radojevic-Skodric S et al. The role of TERT-CLPTM1L SNPs, hTERT expression and telomere length in the pathogenesis of oral squamous cell carcinoma. J Oral Sci 2016;58:449-58
22. Fernández-Marcelo T, Sánchez-Pernaute A, Pascua I et al. Clinical relevance of telomere status and telomerase activity in colorectal cancer. PLoS One 2016;11:e0149626.
23. Jafri MA, Ansari SA, Alqahtani MH, Shay JW. Roles of telomeres and telomerase in cancer, and advances in telomerase-targeted therapies. Genome Med 2016;8:69.
24. Pal D, Sharma U, Khajuria R, Singh SK, Kakkar N, Prasad R. Augmented telomerase activity, reduced telomere length and the presence of alternative lengthening of telomere in renal cell carcinoma: plausible predictive and diagnostic markers. Gene 2015;562:145-51.
25. Buseman CM, Wright WE, Shay JW. Is telomerase a viable target in cancer? Mutat Res 2012;730:90-7.
26. Bell RJ, Rube HT, Xavier-Magalhães A et al. Understanding TERT promoter mutations: a common path to immortality. Mol Cancer Res 2016;14:315-23.
27. Wang K, Liu T, Liu L et al. TERT promoter mutations in renal cell carcinomas and upper tract urothelial carcinomas. Oncotarget 2014;5:1829-36.
28. Casuscelli J, Becerra MF, Manley BJ et al. Characterization and Impact of TERT Promoter Region Mutations on Clinical Outcome in Renal Cell Carcinoma. Eur Urol Focus 2017 Sep 24 (epub ahead of print).
29. Nguyen D, Taheri D, Springer S et al. High prevalence of TERT promoter mutations in micropapillary urothelial carcinoma. Virchows Arch 2016;469:427-34.
30. Zheng X, Zhuge J, Bezerra SM et al. High frequency of TERT promoter mutation in small cell carcinoma of bladder, but not in small cell carcinoma of other origins. J Hematol Oncol 2014;7:47.
31. Diaz A, Puig-Butillé JA, Valera A et al. TERT and AURKA gene copy number gains enhance the detection of acral lentiginous melanomas by fluorescence in situ hybridization. J Mol Diagn 2014;16:198-206.
32. Xie H, Liu T, Wang N et al. TERT promoter mutations and gene amplification: promoting TERT expression in Merkel cell carcinoma. Oncotarget 2014;5:10048-57.
33. Vinci S, Giannarini G, Selli C et al. Quantitative methylation analysis of BCL2, hTERT, and DAPK promoters in urine sediment for the detection of non-muscle-invasive urothelial carcinoma of the bladder: a prospective, two-center validation study. Urol Oncol 2011;29:150-6.
34. Nikolic N, Carkic J, Ilic Dimitrijevic I et al. P14 methylation: an epigenetic signature of salivary gland mucoepidermoid carcinoma in the Serbian population. Oral Surg Oral Med Oral Pathol Oral Radiol 2018;125:52-8.
35. Haraguchi K, Yada N, Sato S et al. The methylation status and expression of human telomerase reverse transcriptase is significantly high in oral carcinogenesis. APMIS 2017;125:797-807.
36. Davis CF, Ricketts CJ, Wang M et al. The somatic genomic landscape of chromophobe renal cell carcinoma. Cancer Cell 2014;26:319-30.
37. Chen Z, Wang J, Bai Y et al. The associations of TERT-CLPTM1L variants and TERT mRNA expression with the prognosis of early stage non-small cell lung cancer. Cancer Gene Ther 2017;24:20-7.
38. Shen N, Lu Y, Wang X, Peng J, Zhu Y, Cheng L. Association between rs2853669 in TERT gene and the risk and prognosis of human cancer: a systematic review and meta-analysis. Oncotarget 2017;8:50864-72.