Reviewer's report

Title: Expression and biological-clinical significance of hTR, hTERT and CKS2 in washing fluids of patients with bladder cancer.

Version: 2 Date: 27 July 2010

Reviewer: Daniele Calistri

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The paper focuses on the investigation of the role of hTR, hTERT and CKS2 in bladder cancer (BC) carcinogenesis. In addition, significant changes are observed in the expression levels of these three genes, the authors evaluate their role as markers of diagnosis and progression. This is a case-control study based on the evaluation of these markers in bladder washings. The authors find a significant decrement of hTR in superficial BC, becoming more evident in invasive BC. Conversely, they highlight an upregulation of hTERT and CKS2 in both cancer subgroups. Using a logistic regression model, it is seen that hTR and CKS2 is the best approach in BC diagnosis when compared with the evaluation of single markers.

Finally, hTERT is shown to be one of the markers most capable of identifying BC superficial forms, whereas hTR is the best one for highlighting the BC invasive form.

Minor Essential Revisions:

Introduction
References 17-22, cited on page 4, are not only related to articles that deal with hTERT mRNA expression only but also to telomerase activity. The text should be changed to reflect this.

Material and methods
Were the patients at the first diagnosis of BC? Were there any cases of CIS in the superficial cancer group?

The authors state that controls comprised individuals with no history of BC and also individuals undergoing follow up for BC. This population could perhaps have been better selected.

Page 5, line 4: what were the clinical indications of the control subgroup; are the authors referring to hematuria/irritative symptoms or a previous history of cancer?

Results and Discussion
There were 36 patients with bladder cancer; among these, 26 had a superficial low-grade tumor and 10 high-grade invasive cancers. The series is probably too small to permit correct statistical comparisons between these two subcategories.
The approach proposed in bladder washing is invasive and it is not clear what the final aim of the authors is for the schedule proposed. Why do the authors not try to evaluate the accuracy of these biological markers in voided urine?

The expression of hTR is lower in cancer patients than in healthy individuals and, among tumors, in high-grade than in low grade lesions. The authors discuss this aspect in the third paragraph of the Discussion but it would be an interesting to compare their findings with studies in the literature, which, conversely, observe an increased expression of telomerase subunits in cancer patients compared to healthy individuals.

**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.