Author's response to reviews

Title: Short rare hTERT-VNTR 2-2nd alleles are associated with prostate cancer susceptibility and influence gene expression

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Author's response to reviews: see over
Managing Editor, Miss Angelina Ilievska MSc
BMC Cancer
(on behalf of Xin Chen)

June 16, 2010

Dear Editor,

We really appreciate your kind consideration of our manuscript. After reviewing your letter, I am writing on behalf of all co-authors to ask you to reconsider your decision. We have addressed the specific concerns detailed in the reviewers' comments.

We would like to resubmit our manuscript entitled "Short rare hTERT-VNTR 2-2nd alleles are associated with prostate cancer susceptibility and influence gene expression" (BMC Cancer, paper No. 1193289226362849) as a Research Article in BMC Cancer. This manuscript has been read and approved by all listed authors.

We would very much appreciate it if you would reconsider our manuscript positively.

Sincerely,

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Short rare hTERT-VNTR 2-2nd alleles are associated with prostate cancer susceptibility and influence gene expression.

REFEREE COMMENTS

Reviewer: Ju-Seog Lee

Reviewer's report:
The manuscript is on an association study between one of hTERT alleles (hTERT-VNTR 2-2) and tumor susceptibility in Korean population. In addition to 4 previously known alleles, authors identified 3 rare alleles during screening in large population.

This study is of interest, as genetic association was discovered and confirmed in large size of study population. Authors discovered that shorter repeat alleles are significantly associated with risk of developing prostate cancer, especially with younger patients (<65yrs). Using reporter gene assay, author also demonstrated that expression of hTERT is significantly associated with presence of hTERT-VNTR 2-2 only in prostate cancer.

Particular Suggestions regarding the manuscript (Minor comments)
1. Please describe how volunteers who were selected as control subjects were recruited.

- We included the following sentence in Materials and Methods.

Controls were selected from the Department of Preventive Medicine and Internal Medicine of Dong-A University hospitals between 2000 and 2004 (Busan, Korea). A total of 421 male individuals in the control group who had no personal history of cancers or current cancer were recruited and completed an interview.

2. It is interesting to see expression of reporter genes with promoter containing tandem repeat is significantly higher in prostate cancer cell line, supporting author’s hypothesis. But it might also useful for readers if authors include some alternative possibility in discussion.
- We hypothesize that these short rare hTERT-VNTR 2-2nd alleles may indicate susceptibility to prostate cancer by a case-control study and luciferase assays revealed that the reporter construct containing the minisatellites increased the activity of luciferase in PC3 and LNCap prostate cancer cell lines. Indeed, this finding may be indicative of the limitations of in vivo transcriptional regulation, though the high density of putative binding sites within the repeat array for transcription factors such as ER may enhance TERT gene expression.
- We included the following sentence in the discussion: When we examined the luciferase activity of the TERT promoter that included the VNTRs in gastric cancer, no increase was detected (data not shown). Therefore, the high density of putative binding sites within the repeat array for transcription factors such as ER may enhance TERT gene expression with cell line specificity.

3. no major concerns on statistical analysis

Reviewer: Francesca Demichelis

Reviewer's report:
Yoon et colleagues investigated the association between variable number tandem repeats (VNTRs) in intronic regions of hTERT and increased risk of developing prostate cancer in a case-control study with individuals recruited from five different city hospitals in Korea. They report on three novel minisatellite alleles in the hTERT-VNTR 2-2nd. They detect a five-fold odds ratio of association for rare alleles versus common alleles with rare allele rate of 1.22% in cases in 0.24% in controls. Finally, they assessed the transcriptional levels of a TERT promoter-driven reporter gene in the presence of VNTRs with a varying number of repeats in prostate cancer cell lines.

Major Compulsory Revisions

What is the effect of testosterone or estrogen cell treatment on hTERT promoter expression?
- According to previously published results [Kyo et al. (1999, Cancer research); Calado et al. (2009, Blood)], two ER positions at -2677 and -873 are reported. But, the hTERT promoter used in our study is located from – 304 to + 40 (Hotokawas et al., 2002 Mol. Biol. Cell). Moreover, when we studied the luciferase activity with TERT promoter alone (pBT304, without VNTR sequence), it showed the same levels of luciferase activity in control and prostate cancer cell lines.
We found high density of putative binding sites within the repeat array for transcription factors ER. The estrogen receptor α subunit is expressed in androgen receptor-dependent prostate cancer, suggesting that the ER pathway may be involved in prostate cancer.

The authors should consider the evaluation telomere length and assess their associations with respect to the common and the rare alleles. What is the expected relation between hTERT expression and telomere length?

- Actually, for the case-control study we used genomic DNA from whole blood. As you know, these genomic DNAs could check the genotype, but it couldn’t reflect the cancer situation. We don’t have any cancer tissues with rare alleles to assess the telomere length.

- As you know, previous study [Harley et al. 1990. Nature; Counter et al. 1992. EMBO; Meyerson et al. 1997. Cell] reported that the activation of telomerase appears to be a major step in the progression of human cancers. Unlike normal human cells, cancer cells can be established as permanent cell lines and thus are presumed to have undergone immortalization during the process of tumorigenesis. It was reported that high levels of telomerase activity result in continuous telomere elongation [Cristofari and Lingner (2006, EMBO J); Hug and Lingner (2006, Chromosoma)]. In super-telomerase HT1080 cells, telomerase activity was increased 40 times and telomere-length increased from 2.5 to 20 kb in 50 PD (eight-fold) [Cristofari and Lingner (2006, EMBO J)].

- As we mentioned, hTERT-VNTR 2-2nd is located in an intron and we speculate that there is an association between the short rare hTERT-VNTR 2-2nd variants and prostate cancer. We hypothesize that these short rare hTERT-VNTR 2-2nd alleles may indicate susceptibility to prostate cancer by a case-control study and luciferase assays revealed that the reporter construct containing the minisatellites increased the activity of luciferase in PC3 and LNCap prostate cancer cell lines, but with no clear relationship between the number of repeats (common vs rare) and the change in expression. Indeed, this finding may be indicative of the limitations of in vivo transcriptional regulation, though the high density of putative binding sites within the repeat array for transcription factors such as ER may enhance TERT gene expression.

WHO classification system needs to be used for the characterization of the prostate cancer cases.

- According to referee’s comment, we remade Table 3 for the clinicopathological information and included the tumor grade of cancer cases in Table 5 for additional information for prostate cancer cases.

The study results are limited in clinical applicability due to the very low rate of associated alleles and warrant external validation. The authors should address these points in the discussion and tone down the sentence about usability as diagnostic biomarker of increased risk for prostate cancer and cancer progression.

- There are many reports of altered TERT expression in various cancers, but no genetic regulation by VNTRs is known. Our result could not explain directly what kind of mechanism is involved in TERT upregulation in the prostate cancer cell lines. But, we suggest that the minisatellites influence gene function and it may be used as a marker for genetic association.

- According to referee’s comment, we changed the discussion and included the following sentence in the Discussion.

  This finding may prove useful as a diagnostic biomarker of increased risk for prostate cancer and...
cancer progression, though the short rare alleles group is too small to be common in prostate cancer cases.

Minor Essential Revisions
Table 2 does not seem to add anything to the information included in Table 1.
- We remade Table 1 and Table 2 and changed the order of Tables 1~3 for better understanding. Table 1 focuses on the distribution of each haploid allele and compares between controls and cases for allelic distribution. Table 2 represents the analysis of genotype frequency (2 alleles in each individual) between controls and cases. We remade Table 2 with the additional information of comparisons between C/C (included common and common alleles) and C/R (included common and rare alleles) in controls and cases.

Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:
I declare that I have no competing interests

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