

Rome, 23.06.2014

Dear Editor,

Thank you for having revised the article (ESPS Manuscript NO: 10639) entitled “Human Papillomavirus has not a causal role in colorectal carcinogenesis” submitted to World Journal of Gastroenterology.

We have full-addressed all the comments of the reviewers and I hope you will formally accept this revised version of our manuscript for publication.

Enclosed with this letter you will find a point-by-point response to the comments we received.

For the Editor:

1. We included sub-heading regarding the Article Type for your Colum Journal in the Title page as follows: *Article Type*: Research Report Case-Control Study
2. We specified the academic title of the corresponding author and included in the Title Page the authors' contributions
3. We submitted the manuscript for professional editing (including in this re-submission AJE Certificate)
4. We included the Figures as Power Point files for journal's editing

Reviewer 1:

Q1. I ask authors to describe about the gene characteristics (genotype and quantification data) of HPV in other HPV-related cancers (cervical cancer and oropharyngeal cancer) and discuss by comparison with your data of CRC.

A.1 A comment has been added in the **Discussion** section in the light of the reviewer's suggestion referring to the papers by Munoz and Hafner (for cervical cancer) and Gillison and co-authors for the oropharyngeal cancer, as follows: “Notably, the reported HPV prevalence of 16.9% in the colorectal cancers we examined is much lower than that of other HPV-associated cancers, as the prevalence of HPV in cervical cancer has been reported to be approximately 96% ^[27], and approximately 30-44% in oropharyngeal cancers ^[28].”

Chaturvedi et al. recently investigated a large series of oropharyngeal carcinomas using the Inno-Lipa technique; they determined that the HPV prevalence in oropharyngeal tumors was 44.1% (38.8% for HPV16). Among the 102 Inno-LiPA HPV16-positive tissues, 77.5% were positive based on viral load criterion, with a median of 22.1 HPV16 copies/cell, and 84.5% were positive by viral oncogene expression criterion, with a median of 152.9 transcripts/1,000 *RPLP0* equivalents ^[28].

Similar findings were reported in the investigation of HPV16 in cervical cancers; notably, Hafner et al. reported viral integration as a key event in malignant lesions: they found integrated HPV16 virus in 51% of cervical cancers tested by APOT assay. Conversely, just 11% of CIN lesions contained HPV16 integration.

Furthermore, viral transcript levels were broadly distributed, though they had similar median values irrespective of histo-pathological grading and physical state of the viral genome both in CINs and cancers [11].”

Q2. Page 6, 7th line: Please correct from “froze” to “frozen

A2. Materials and Methods were revised according to the reviewer suggestion

Reviewer 2:

Q1. Page 7 and 8: what is the CT method?

A1. Δ CT method has been clarified in the **Materials and Methods** as follows: “ Δ CT method (CT target-CT reference) referring to the episomal cell line (W12), considered equal 1.”

Q2. Tables: what does VLN mean Tables: Grading of CRC had only 3 grades, this needs to be corrected
TNM: what TNM classifications did the authors use?

A2. A footnote clarifying V/L/N acronym has been added to the **Tables** as follows: “*V/L/N: perivascular/lymphovascular/perineuralinvasion; Garding has been corrected and AJCC classification specified.” Of note, none of our patients has been staged T4bN0

Q3. Transcriptional inactive = transcriptionally inactive

A3. The typographical error has been corrected through-out the manuscript

Q4. The following statement is unclear: This could be related to the very low amounts of viruses or to a different target epithelium that could bias a productive infection or the transcriptional patterns of the HPV

A4. This sentence has been better stated in the **Discussion** section as follows: “Our negative results could be explained by the very low virus quantities in positive tissues or by different transcriptional patterns of the virus in a different target epithelium that could impair viral protein replication and expression (simple cuboidal in colorectum vs. squamous stratified in the cervix). “

IMPORTANT: I noticed that on the Download dashboard of the EPS web page there is an invoice for the publication fees. However, according to the email that I received from your office on December 2013 by Lian-Sheng Ma, President and Company Editor, I was accepted as a member of the Editorial Board for the World Journal of Gastroenterology, thus I should have all the fees waived. Editor Number ID:00181214

Sincerely

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