Author's response to reviews

Title: Evaluation of high-risk Human papillomaviruses type distribution in cervical cancer in Sichuan province of China

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Author's response to reviews:

Dear Editor:

Thank you very much for your letter regarding our manuscript entitled “Evaluation of high-risk Human papillomaviruses type distribution in cervical cancer in Sichuan province of China.” We have addressed the comments carefully and revised the manuscript point-by-point as indicated below.

REFEREE #1

1. The sentence “The nested TS PCR showed greater sensitivity than the one-step TS PCR for detection of HPV types 16, 52, 58 and 59; this increased the positive rate of these types greatly and identified many more multiple infections.” at beginning of 3 rd paragraph in Discussion is changed as “Combination of one-step TS PCR and Nested TS PCR increased the positive rate of HPV types 16, 52, 58 and 59 greatly and identified many more multiple infections.”, and in Table 3(Table 2 in revised manuscript), “Nested TS PCR” is changed as “Combination of One-step TS PCR and Nested TS PCR”

2. Following the suggestion of the reviewer, we described the primers of one-step PCR first in Table 1.

3. Many of HPV50’s infections detected in this study were co-infected with other high risk HPV types, which have higher viral load than the HPV 50’s; we agree that in these specimens, the HPV 50’s might play a secondary or unimportant role in the progress of development of cervical cancer. However, the viral loads of the HPV 50’s in some co-infection specimens are equal or higher than those of the HPV 16 or 18 in this study.
To data:

- Both types were detected by one-step PCR in 1 case of HPV 16/52 and 1 case of HPV 16/59.

- All types were detected only by nested PCR in 4 cases HPV 52/16, 8 cases HPV 58/16, 2 case HPV 59/16, 1 case HPV 16/18/58, 1 case HPV 59/16/18.

- The HPV 50's were detected by one-step PCR and HPV 16 was detected only by nested PCR in 1 case HPV 52/16, 4 cases HPV 58/16, 3 cases HPV 59/16 (These data were not shown in manuscript.).

We feel that the role of HPV 50’s in these specimens in progress of development of cervical cancer can’t be neglected. Besides, the detection rates of HPV 50’s in this study are rather higher than other regions; the high prevalence of HPV52, 58 and 59 in cervical cancers in this area should be considered in designation of HPV screening systems and development of HPV vaccines in China.

REFEREE #2

Abstract

• The conclusion has been improved:

Our data demonstrate that, besides HPV 16, which was found to be the most prevalent type, HPV types 58, 52 and 59 are more prevalent than HPV18 in women with cervical cancer in the Sichuan area of China.

Background

• Approximate numbers have been used in the first paragraph:

Cervical cancer is the second most common cancer in women; each year approximately 500,000 women worldwide are diagnosed with invasive cervical cancer and more than half of them die of this disease [1]. Eighty percent of these deaths occur in developing countries. In China, there is an annual incidence of about 46,000 cases, and cervical cancer presents a major health problem [1].

• PCR has been defined in the second paragraph:

The most widely used method for HPV detection in cervical cancers is based on polymerase chain reaction (PCR) using either consensus or type-specific primers for the amplification of HPV DNA.

Methods

• All dots after the subtitles have been deleted and each assay in subtitles has been changed to singular.
• The catalog number of the DNA extraction kit has been deleted:
DNA was extracted from frozen tissue specimens by DNeasy Tissue Kit (QIAGEN), according to the manufacturer's instructions.

• The paragraph “MY09/11 PCR assay” has been placed before “One-step TS PCR assay”.

• We have included a reference for the primers used in the One-step TS PCR assay:
All specimens were tested by one-step TS PCR assays using type-specific primers for HPV types 16, 18, 52, 58 and 59, which were previously described by Sotlar et al. [17] (Table 1). The PCR was performed …

• The paragraph referring to Nested TS PCR assays has been corrected per the reviewer's suggestions:
To increase the sensitivity of the detection for HPV16, 18, 52, 58 and 59, the specimens negative by the One-step TS PCR for any genotype were further amplified by Nested TS PCR. The TS primers for HPV types 16, 18, 52, 58 and 59 of the first round of amplifications were designed specifically for this study to amplify a wider region (ranging 619 to 662 bp) than those targeted by primers used in the One-step TS PCR assay, which were used in the second round of amplification of the Nested TS PCR (Table 1).

• The sentences “…according to the consensus primer target region of NMPCR as previously described by Sotlar et al. [17], derived from the E6/E7 oncogenes of HPV types 16, 18, 52, 58 and 59, respectively, producing an amplicon of 619 to 662 bp (Table 1). The second round amplifications used the primers from the one-step TS PCR.” have been deleted.

• Subtitles and footnotes of Table 1 have been corrected:
Primers for the One-step TS PCR and the second round of the Nested TS PCR*  
Primers for the first round amplifications of the Nested TS PCR**

*designed by Sotlar et al. [17] ; **designed specifically for this study

• The DNA sequencing section has been moved and now follows the “Nested TS PCR assay” and precedes “Statistical analysis”, and the name and company of the kit and sequencer used for sequencing has been indicated.

Discussion
• The number of the reference [17] has been inserted after Sotler et al. on page
11, line 1 (page 11, line 2 in revised manuscript).

- The word “employing” has been deleted on page 10, line 7 (page 10, line 8 in revised manuscript).

- The sentence on page 10, line 12 (page 10, line 13 in revised manuscript) has been corrected as follows: “…and it is difficult to cover all HPV types that could be found in precancer and cervical cancer [6-8].”

- The “to” has been inserted on page 11, line 9 (page 11, line 10 in revised manuscript).

- On page 11, line 12 and 13 (page 11, line 13 and 14 in revised manuscript), the sentence has been replaced with “Therefore, the TS primers for HPV type 16, 18, 52, 58 and 59 for the first round of amplification of the Nested TS PCR assay were specifically designed for this study.”

- All capitalization of “one-step TS PCR” and “nested TS PCR” has been changed to “One-step TS PCR” and “Nested TS PCR.”

- The footnote on Table 3 has been deleted.

- Table 3 has been moved before Table 2 and its subtitles have been changed to “HPV detection by the MY09/11 PCR, the One-step TS PCR and the Nested TS PCR in cervical cancer specimens (N=190).”

Thank you very much in advance for your work.

Sincerely yours,

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