Author's response to reviews

Title: Induction of lung lesions in Wistar rats by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and their inhibitions by aspirin and phenethyl isothiocyanate.

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Author's response to reviews:

Dear Editor:
I have finished my revised paper, and resubmitted online, the following is the point-by-point answers to the questions. The writing and grammatical errors have been corrected by a native-speaking English colleague.

Reviewer: PRAMOD UPADHYAYA
Answer to minor essential revisions
1) Question 1 and 2: page 2 line 3,7
They have been corrected in abstract.

2) Question 3: page 7 line 20
The reference has been added

3) Question 4: page 8 line 10
Quote the reference on page 8

4) Question 5: page 9 line 3
Add the reference on the page

5) Question 6: page 9 line 11
Explanation is given on page 9 line 11-12. Normal diet was produced by Laboratory of Wuhan University on the basis of rat formula feeds, which were regulated by standard GB 14924--2001 published by People's Republic of China. The standard regulates the content of components, including protein, fat, amino acids, vitamins, trace elements, choline and so on.

6) Question 7: page 9 line 12
Body weight and food consumption are given in result (figure 9).

7) Question 8: page 13 line 9
Group 1, 2, 3, 4, 5 change to A, B, C, D, E

8) Question 9: page 14 line 12-14
The % decreased atypical dysplasia include in table 1

9) Question 10: page 14 line 21-22
Add explanation on page 14

10) Question 11: page 16 line 3
Add the reference on page 16

11) Question 12: page 33 table 2
Provide explanation in discuss on page 33
Reviewer: Katsumi Imaida
Answers to major compulsory revisions
The reviewer's questions are mainly about whether there are inflammatory changes during the experiment, so I give the combination answer to all three questions.
In theory, instillation may cause inflammatory changes. However, in order to simulate the way of tobacco smoke inhalation by human, we still used the special way and tried our best to minimize the influence of inflammation in the current study. First, all of the instruments used in our experiment were strictly sterilized, and the iodized oil with or without NNK/MCA was limited to no more than 0.1ml. Second, after instillation we usually gave rats streptomycin 250mg/kg body weight and penicillin 100000 U/kg body weight by muscle injection for 7 days to decrease the infection. Our previous work showed that these ways effectively decreased the inflammation. Moreover, the treated rats did not show any symptom of respiratory infection during the experiment. Histologically, there was also no obvious inflammation response in major organs of rats. Third, in present results, we observed that the expressions of PCNA and COX-2 varied in different lesions in the same rat (for example normal mucosa, metaplasia, dysplasia, CIS and invasive carcinoma, sometimes more than one changes could be seen in the same rat.). On the other hand, there were significantly different expressions of COX-2 and PCNA between control group and the others, indicating that the differences of protein expression were not due to inflammation because the control group was treated with vehicle in the same way. So, the results of our previous and present studies strongly support the conclusion that COX-2 and PCNA are induced mainly by tumorigenesis not inflammation in our study, and aspirin+PEITC suppress the progressing directly.
It is very difficult to distinguish between inflammatory changes and tumorigenic proliferation by histological changes. It has been shown that reactive pneumocyte hyperplasia can be seen in various types of lung injury, and the degree of reactive cytologic atypia can even exceed the neoplastic atypia of some lung cancers. In the first part of our study, the incidence of squamous cell carcinoma reached 56% in rats treated with MCA, so I don't think it is doubt that metaplasia, dysplasia and CIS observed in this group are the early stages of tumorigenesis. Although, in present study, we only got preneoplastic lesions in rats treated with NNK, we realize that these lesions are mainly induced by NNK directly, for the reason mentioned above. Now, we are carrying out long-term study to prove these lesions will in fact lead to adenocarcinoma. This may be the most important to confirm our data.

Answers to minor essential revisions
1) Question 1: page 4 line 5
"F344" has been deleted.

2) Question 2: page 4 line 3
"Wister" has been changed into "Wistar".

3) Question 3:
The explanation has been given in discussion page 14.

4) Question 4: page 9 line 3 and 6
Use the same expression, "x40".

5) Question 5: page 13 line 9-16
Use the same effective digits of the numbers of percentage.

Reviewer: Myung-Haing CHO
Answers to major compulsory revisions
1) Question 1:
In present study, we established the protocol to inducing preneoplastic lesions in Wistar rat by a single intratracheal instillation of NNK. We discuss clearly that our model is better than others for studying lung cancer in page 14-15. We think it could be a good model for exploring the differences between squamous cell carcinoma and adenocarcinoma of lung, taken together with our previous model of squamous cell carcinoma induced by MCA. We know the chemopreventive effects of aspirin and PEITC have been studied extensively by other researchers, but it is poorly understood about the effect of aspirin and PEITC on early stage of tumorigenesis and the effect of PEITC on post-initiation way. It is very important that chemoprevention should be used in the early stage of tumorigenesis, so our aim is to declare whether aspirin and/or PEITC have effects in early stage of tumor progression.

2) Question 2:
The method of enthanasia added to page 6.
Animal Biosafety Level III Laboratory of Wuhan University was established by Chinese Department of Education and Wuhan University. Conditions of ABSL- of Wuhan University reach the requests of Laboratory animal Requirements of environment and housing facilities (GB 14925-2001) published by P.R.China. It is qualified to study virus with high pathogenicity, such as SARS, HIV. We have approval for production and use of lab animal (rodent, rabbit, canis and simian) from Hubei Province Department of Science and Technology, license No. SYXK()20030013

3) Question 3:
The reference has been given to the methods on page 6

Answers to minor essential revisions
Question 1 and 2
The writing and grammatical errors were corrected by a native-speaking English person.

Regards
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