Author's response to reviews

Title: The -271 G>A polymorphism of kinase insert domain-containing receptor gene regulate the gene's transcriptional level in the patients with non-small cell lung cancer

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Author's response to reviews:

Dear Joseph Dunckley,

Thank you for your careful, knowledgeable, and kindly reviews on our manuscript ("The -271 G>A polymorphism of kinase insert domain-containing receptor gene regulate the gene’s transcriptional level in the patients with non-small cell lung cancer"). We have revised our manuscript according to your comments. The responses to the comments were as follows:

Reviewer: Rodney J. Scott

Major compulsory revisions:

1. For accurate frequency estimates of the -271 G>A KDR polymorphism the authors must use a local population based sample set of at least 200 healthy controls. It is not good enough to examine a website and make inferences from it. This will provide better information about the importance of this polymorphism in relation to lung cancer progression.

Response: We detected 203 healthy controls from a pool of healthy volunteers who visited our hospital's check-up center. Genomic DNA of the control group was isolated from the lymphocytes of whole blood samples using the Universal Genomic DNA Extraction kit (TaKaRa, Dalian, China). There was no significant difference in distribution of the genotype of the KDR gene polymorphism between the lung cancer patients and the control group (x²=1.269, P=0.264, Fisher's exact test). We have added this information into the revised manuscript.

2. The clinical features comparison made in Table 1 is incorrect. The comparison is between patients with the AA genotype against the GA/GG genotype - this means that there are for example 59% males with the AA group against 62% males with the GA/GG genotypes. This must be re-calculated for the entire table.
Differences between the groups should then be determined using Fishers exact test.

Response: We have re-calculated for the entire table. Differences between the groups have also been determined using Fishers exact test in the revised manuscript.

3. The observation that there is no difference in the protein levels of the KDR protein irrespective of the mRNA levels must be explained further. The discrepancy between the finding could be due to more efficient clearance of the mRNA species (ie mRNA degradation) rather than the polymorphism being related to the promoter activity of the gene.

Response: We have explained further of this discrepancy between RNA and protein in the "Discussion" section of the revised manuscript. (This inconsistency of mRNA (transcription) and protein (translation) is probably due to the complex of gene expression and the multi-stage regulation mechanisms of the gene. That maybe correlated with the more efficient clearance of the mRNA species (ie mRNA degradation). Protein can not only be regulated on transcriptional level but also translational level and turn over level. It is also possible for the protein to be regulated by different turn over rate and regulated post-translationally.)

Reviewer: Heidi Greulich

1. The authors of this manuscript have identified a polymorphism in the 5' UTR of the receptor tyrosine kinase gene KDR and claim to have found a correlation with mRNA expression. The discovery and frequency assessment of the polymorphism seems sound. Conclusion of association with lung adenocarcinoma in Asian patients seems warranted, based on their reported frequency of the AA genotype in this population from dbSNP. Is it possible to get a P-value for this association (Fisher Exact, perhaps)? This correlation should be emphasized more, whereas the reported "marginal significant" associations with age and smoking status seem unnecessary.

Response: It is more interesting if we can get a P-value for this association (Fisher Exact, perhaps), but the sample size is not shown in the dbSNP. So, we detected 203 healthy controls from a pool of healthy volunteers who visited our hospital's check-up center and added this information into the revised manuscript. We have deleted "marginal significant" associations with age and smoking status in the revised manuscript.

2. The mRNA expression analysis also seems sound. It should be clarified as to whether these are real-time PCR results (not clear from the manuscript). However, it is hard to imagine many scenarios in which an increase in mRNA levels that does not affect protein levels is physiologically relevant. Also, the positive and negative IHC photos supplied as examples do not look so different to my eyes. They also appear to be presented at different magnifications. Would it be possible to supply a series of photos for each category taken at the same magnification?
Response: We have added the "real time PCR" into the title of results. And we also changed another series of photos for each category (negative and positive) to the revised manuscript. We have discussed more deeply in the revised manuscript about the scenarios in which an increase in mRNA levels that does not affect protein levels.

Thank you.

Very Respectfully,

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