Reviewer’s report

Title: ICOS gene polymorphisms are associated with sporadic breast cancer: a case-control study

Version: 2 Date: 3 July 2011

Reviewer: Ke-Da Yu

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Major Compulsory Revisions

In Abstract
1. The nomenclature of SNP is inconsistent. The five polymorphisms (rs11889031, IVS1+173, rs4675374, c.602 and c.1624) were addressed using either the RS number or the gene location. The SNP such as IVS1+173 has a RS number in the NCBI db-SNP. Please revise the SNP name.

2. The difference between haplotype CTCAC and CCCAC is just due to the differential SNP of IVS1+173 (T and C). Does the haplotype analysis make sense?

2. The results have no data regarding survival. How the authors state that the “ICOS gene polymorphisms may affect the prognosis of breast cancer”. It is over-interpreted and the conclusion should be revised.

In Methods
1. Please provide the representative electrophoresis plots of PCR-RFLP.
2. How did authors choose these five SNP? What is the rationale?
3. No statistical power analysis was conducted. How did they determine the sample size?
4. Before performing PCR-RFLP, they should have evaluated the accuracy of this method. PCR-RFLP is likely to be false-negative or false-positive if unsuitable primers and restriction enzymes were selected. Were the PCR-RFLP results consistent with sequencing results? How consistent?
5. What is the relationship between the five SNPs? Are they in LD?

In Results
1. The haplotype analysis is somewhat weird. Does the combination of the five SNPs represent the haplotype of the ICOS gene? What the original mean of haplotype in genetics?
2. How did they define the positivity of ER, PR, HER2, and p53?
3. The results were not adjusted for other risk factors (but only age) of breast cancer.
4. No demographic information is presented for controls; no baseline comparison
was performed between cases and controls.

Table 1
The positive rate of HER2 is high up to 30%, which is higher than previous data (about 20-25%).

Table 2
No multiple-comparison correction is conducted. Are so-called “significant” results only because of false-positive?

Minor Essential Revisions
1. Why the “IFN-#”, “ICOS”, “CTLA-4” in sentence “population produced higher IFN-#”, indicating that ICOS interacts with CTLA-4 and plays an important role in tumor immunity” were all in italic. They are protein rather than gene in the context.
2. In Supplemental Table 1, the results are incomplete.
3. Table 3, how were the P-values calculated?

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

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