Reviewer's report

Title: The NFKB1 polymorphism (rs4648068) is associated with the cell proliferation and motility in gastric cancer

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Reviewer: Kirsten Stone

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The authors attempt to answer an important and interesting question about the mechanism of NF#B polymorphism - dependent differences in gastric cancer incidence. Data are presented to show that expression of NF#B is correlated to genotype; other results show increased binding of C/EBP# to the NFKB1 promoter and increased luciferase activity in the presence of the GG genotype. In addition in vitro data are presented that tie these results to an increased proliferation and migration rate as well as a decreased apoptosis rate. These data would be an important contribution if they were more convincing.

Major Compulsory Revisions

1. Figure 1 shows NF#B immunohistochemistry of tissues from gastric cancer patients. However, the actual origin and tissue type is not described in either methods or results. Quantification is presented but it is not clear what the denominator is. Is it all nuclei in the tissue? Were multiple sections from multiple patients evaluated? Also, the presented tissue sections are not comparable between genotypes. There seem to be different tissue types in each panel. Panel c) shows mostly connective tissue and fat, b) epithelial tissue in one panel, connective tissue in the other, and a) a mix of tissues with what appears to be mostly lymphocytes in the right hand panel. The method section provides a formula but does not describe how positive cells were counted. If subjective measures were used, the intensity of staining may be variable between slides and not necessarily indicative of quantity. How was the statistical analysis done? A better approach to quantify the expression of NF#B in gastric cancer tissue is using qPCR and/or western blot analysis.

2. Figure 3 includes a nice approach to show the role of NFKB polymorphisms in transcriptional activity. However, the result section should be expanded to provide a clear description of all panels and a summary of the results of these experiments in both non-gastric and gastric cancer cell lines. For data presented in table 1, it is not clear which luciferase plasmid was used. If the description in the legend means that the data presented are the difference between luciferase activities of GG and AA reporters, it should be clearly described in the results section.

3. Figure 5 provides data supposed to demonstrate the physiological importance of the described effect. However, there are several problems with the presentation. It is not clear whether the plasmids are stably transfected into cells.
A transient transfection will usually become ineffective within 3 days, so it is not clear whether the cells still contained the constructs after 6 days. More importantly, it is not clear to me how transfected promoter regions regulating a luciferase gene can change the transcription of endogenous NFkB target genes. The authors should explain how that works and provide evidence from the literature that this is a valid approach.

**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:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests.