Reviewer's report

Title: The NF-kB p65 and p50 Homodimer Cooperate with IRF8 to Synergistically Activate iNOS Transcription

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Reviewer: Tomohiko Tamura

Reviewer's report:

This manuscript describes that the cooperation between TNFα-NF-κB and IFNγ-STAT1-IRF8 pathways is important for the expression of iNOS in both colon carcinoma cells and myeloid cells. The results contain novel findings and are important for the understanding of cancer biology.

Major Compulsory Revisions

1. Several complementary experiments are required to conclude that both colon carcinoma and myeloid cells share the same regulatory mechanism for iNOS expression.
   a) In Figure 4C, the NF-κB/DNA complex in TNFα+IFNγ-treated T84 cells seems to be totally supershifted by a p65 antibody (although due to the red line, the bands cannot be seen well). This would suggest that unlike in myeloid cells, this complex in tumor cells does not contain a p50/p50 homodimer. To conclude that the NF-κB complex in tumor cells is also the NF-κB “homodimer” (in this case p65/p65) like in myeloid cells, a p50-specific antibody should be tested to show that the band is not shifted. Please include a positive control for supershift by the p50 antibody.
   b) In Figure 7A, the authors found that IFNγ induced IRF8 expression in J774 cells. Whether IFNγ induces IRF8 in T84 cells should be examined.
   c) In Figure 7B, in order to conclude that IRF8 is required for the induction of iNOS expression in myeloid cells, the authors should show that the re-introduction of IRF8 into CL-2 cells can restore the induction of iNOS. This is because J774 and CL-2 cells are not siblings and it is unclear whether the observed difference is indeed due to IRF8. In addition, to conclude that IRF8 is also required for the induction of iNOS expression in colon carcinoma cells, IRF8 should be knocked-down or knocked-out in T84 cells.

2. Several controls should be included to ensure the results from ChIP or IP experiments.
   a) p50-ChIP without TNFα treatment to show that the binding is TNFα-dependent (Figure 4B)
   b) ChIP-PCR for known a STAT1-binding site(s) to show that pSTAT1-ChIP itself is working (Figure 4B and 6B)
   c) p65-ChIP without LPS stimulation to show that the binding is LPS-dependent
(Figure 6B)

d) p50-IP without LPS stimulation to show whether the p65/p50 association is LPS-dependent (Figure 6C)

3. Please state how many times each result was confirmed by separate experiments.

Minor Essential Revisions

1. In line 169, “Table S1” should be “Table 1”.
2. In line 272, “p50-specifc” should be “p50-specific”.
3. In lines 321-322, “has been show to” should be “has been shown to”.
4. In line 581 (the legend of Fig. 3B), “analyzed by real time RT-PCR” should be “analyzed by Western blotting”.
5. In Figure 7B, “FN-#” should be “IFN-#”.
6. Inconsistent spellings:
   a. IFN# and IFN-#
   b. “anti-“ and “Anti-“ for the prefix of antibodies in the Figures.
   c. “p65/DNA” and “p65-DNA”

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

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I have no competing interests.