Reviewer's report

Title: Down-regulation of the expression of CCAAT/enhancer binding protein alpha gene (C/EBPalpha) in cervical squamous cell carcinoma

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Reviewer: Minoru Tomizawa

Reviewer's report:

Down-regulation of C/EBPa in carcinogenesis is a classical idea. C/EBPa in cervical cancer has not been analyzed well. The aim and conclusion of this study is reasonable and understandable.

Major compulsory revisions
1. However, staining results of C/EBPa did not seem consistent. This means that the results were not reliable.
2. Transfection experiments were repeats from many literatures in 1990s. Problem of transient transfection is its efficiency. Non-transfected cells interfere the results. How did the authors think about transfection efficiency? The structure of this manuscript is not well organized.

Minor essential revisions
1. Introduction: First paragraph: Problem should be more clearly explained with cervical cancer. Explain the reason why C/EBPs was analyzed. Were there any suggestive data about the link between cervical cancer and C/EBPs?
2. Methods: Information on a kit for cDNA synthesis is absent.
3. Why did the authors use serum deprived condition?
4. Where did cDNA of C/EBPa come from?
5. Regarding luciferase assay. What reporter plasmids used?
6. Figure 1. Immunostaining methods appropriate? Was titration of the antibodies same? A: Collagen fiber is positive. B,C: cytoplasm is positive. C: Central part is positive, but the peripheral part is negative.
7. Figure 2. CIN3 should be spelled out. Figure 1 shows C/EBPa was positive in well, moderately, poorly differentiated squamous cell cancer. But figure 2 showed carcinoma in site was negative. Figure 1 and figure 2 should be consistent.
8. Figure 3. How was the expression levels of C/EBPa normalized?
9. Figure 4. It seemed transient transfection. Transfection efficiency might be low. How did the author recognize the low efficiency? How did they overcome it? Evidence is absent to show that transfected plasmid worked.
10. Discussion: Paragraph 1, 2, 3 were introduction of C/EBPa. Paragraph 4, 5, 6 were the same as results. What did the authors think about the role of C/EBPa in carcinogenesis of cervical cancer? Were there any therapeutic applications?
**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.