Reviewer's report

Title: Immunological response in mice bearing pulchellin-treated breast tumor

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Reviewer: Khalid Matalka

Reviewer's report:

The authors describe the immunological response of pulchellin on mice-bearing breast tumor. The work is good and interesting; however, there are minor essential revisions should be addressed.

Minor Essential Revisions

First the title needs to be adjusted because as it says that breast cells were treated first with pulchellin and then implanted in mice.

Introduction

Lines 3and 4.. The authors wrote "Cancer immunotherapy aims to stimulate the immune system to destroy tumors by enhancing the production of cytokines and immune mediators..." However, this is one strategy and thus it should be mentioned in the statement above.

Methods:

In the experiment since mice were injected with 0.1 ml of 0.75 µg/kg, the average body weights of mice should be given.

Why the mice were sacrificed after 7 days post pulchellin injection?

Results:

The type of cells with their adhesion molecules that pulchellin enhanced should be mentioned, not just "adhesion and costimulatory molecule expression on macrophages."

Since a non-significant reduction in the number of CD8 cells and dendritic cells, p value should be >0.5 and not < 0.5!

In the cytokine measurement, Figure 3 shows neither IFN# nor IL-10, but instead the authors are showing TNF-# (three times) response curve from PEC macrophages!!

Discussion:

Since the authors did not work on IL-12, it would be difficult to say that IFN-#-induced pulchellin activates macrophages and it is not IL-12-IFN-# activation pathway i.e. it could that pulchellin activates macrophages and the activated macrophages activates Th1 cells. So both possibilities could take
Since also pulchellin induces proinflammatory (IFN-#, TNF-#) and reduce some anti-inflammatory cytokines (TGF-#, IL-6) but not IL-10 or IL-4, it is worth noting inflammation role in cancer development and cancer suppression in the discussion. Also, it is preferable to mention the reduction of TGF-# and IL-6 on Treg and Th17 development in cancer-bearing animals.

Figure 3 needs to include IL-10 and IFN-# instead of TNF-#.

Table 1. The authors should differentiate between the source of cells (i.e. peritoneal vs spleen.

The English Language needs to be proof read.

**Level of interest:** An article of importance in its field

**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'