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UNIVERSITY OF TAMPERE
MEDICAL SCHOOL

Tampere

September 21, 2019

Alisha DSouza, Ph.D.

Senior Review Editor

JoVE

Dear Senior Review Editor DSouza,

We hereby submit our revised manuscript entitled "*Chemically (DMBA-TPA) induced skin carcinogenesis*" to be considered for publication in the *JoVE.*

First of all, we found both the editorial and the reviewers´ comments very insightful, and have revised the manuscript accordingly. We now feel that we have adequately addressed the criticisms of the editor/reviewers and the manuscript has improved in the process. Please find our response to the editor and to the reviewers' comments attached to this letter below. All authors have read and approved the revised version of the manuscript, its content, and its re-submission to the *JoVE*.

I as a corresponding author, on behalf of all the authors, hereby re-affirm that our manuscript presents original research methodology that has not been published and is not being considered for publication elsewhere. The authors declare no conflict of interest. We hope that you find our revised manuscript acceptable for publication in the *JoVE*.

Yours sincerely,

Professor & Chief Surgeon

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Editorial Comments:

• Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammatical errors.

• Protocol Language: Please ensure that all text in the protocol section is written in the imperative voice/tense as if you are telling someone how to do the technique (i.e. "Do this", "Measure that" etc.) Any text that cannot be written in the imperative tense may be added as a "Note", however, notes should be used sparingly and actions should be described in the imperative tense wherever possible.

1) Examples NOT in the imperative voice: 1.2, 1.3

Authors´ reply & action: The chapters 1.2 and 1.3. have been now revised and are in the imperative voice.

• Protocol Detail: Please note that your protocol will be used to generate the script for the video, and must contain everything that you would like shown in the video. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol.

1) **Please include an ethics statement before your numbered protocol steps indicating that the protocol follows the animal care guidelines of your institution.**

Authors´ reply & action: An ethics statement is now included in the revised manuscript.

2) 1.1: Mention animal strains.

Authors´ reply & action: The DMBA-TPA model has been used widely in many mouse and at least in few rat strains. We have addressed the strain-issue accordingly.

• Protocol Numbering: Please add a one-line space after each protocol step.

Authors´ reply & action: The requested one-line spaces have been added to the revised version.

• Protocol Highlight: Please highlight ~2.5 pages or less of text (which includes headings and spaces) in yellow, to identify which steps should be visualized to tell the most cohesive story of your protocol steps.

1) The highlighting must include all relevant details that are required to perform the step. For example, if step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be included in the highlighting.

2) The highlighted steps should form a cohesive narrative, that is, there must be a logical flow from one highlighted step to the next.

3) Please highlight complete sentences (not parts of sentences). Include sub-headings and spaces when calculating the final highlighted length.

4) Notes cannot be filmed and should be excluded from highlighting.

Authors´ reply & action: We have highlighted the key part of the protocol. It forms a cohesive narrative. Some notions have been left outside of the highlight.

• Discussion: JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please ensure that the discussion covers the following in detail and in paragraph form (3-6 paragraphs): 1) modifications and troubleshooting, 2) limitations of the technique, 3) significance with respect to existing methods, 4) future applications and 5) critical steps within the protocol.

Authors´ reply & action: The discussion part of the manuscript has been written according to the instructions provided above.

• If your figures and tables are original and not published previously or you have already obtained figure permissions, please ignore this comment. If you are re-using figures from a previous publication, you must obtain explicit permission to re-use the figure from the previous publisher (this can be in the form of a letter from an editor or a link to the editorial policies that allows you to re-publish the figure). Please upload the text of the re-print permission (may be copied and pasted from an email/website) as a Word document to the Editorial Manager site in the "Supplemental files (as requested by JoVE)" section. Please also cite the figure appropriately in the figure legend, i.e. "This figure has been modified from [citation]."

Authors´ reply & action: The figures presented in the manuscript are original and have not been presented previously.

Reviewers' comments:

Reviewer #1:

The manuscript by Vahatupa et al. presents their version of a well-established experimental protocol for reliable production of squamous skin tumors in mice. This model involves a sub-tumorigenic topical exposure to the carcinogen, 7, 12-dimethylbenz[a]anthracene (DMBA) followed by chronic applications of an irritating plant compound, 12-tetradecanoylphorbol-13-acetate (TPA). The presentation is standard, and can be replicated by novices. However, correction of several omissions should be taken.

1. **Discussion of controls is required. Typically, these are Ace/Ace, Ace/TPA, and DMBA/Ace.**

Authors´ reply: It has been demonstrated that both DMBA and TPA are needed for the papilloma induction and the chemicals needs to be administered in right order (DMBA first). As the consensus exists on a need to reduce the animal experiments, we cannot advocate the use of multiple controls as it would increase the number of animals needed. However, the effects of a genetic modification in a naïve setting (no chemical exposure) is mandatory step.

Authors´ action: We have discussed the potential controls based on the existing scientific evidence.

2. **A discussion of mouse numbers in a typical experiment is required.**

Authors´ reply and action: Please see "1. Experimental animals, reagents and equipment", where we provide estimates on the number of animals needed to carry out the model in reproducible and reliable manner.

3. The authors should discuss how to apply the DMBA/TPA model to a new existing strain or to a new strain of genetically engineered mice.

Authors´ reply: The DMBA-TPA model should be carried out in genetically engineered mice using wild type (WT) mice as a control. WT mouse strain provides a known standard in terms of susceptibility to tumor formation. Furthermore, it is also crucial to test both genotypes in untreated setting to expose potential differences that might exist between the strains due to genetic modification.

Authors´ action: We provide the framework for carrying out the DMBA-TPA model in genetically engineered mice based on using wild type (WT) mice and untreated animals from both genotypes as controls. Please see discussion, lines 246 – 254.

4. Metastasis of squamous cell carcinomas to skin draining lymph nodes and to lung is not as infrequent as the authors suggest, particularly in strains such as FVB/n or sensitive outbred stocks such as CD-1 or SENCAR.

Authors´ reply and action: We thank reviewer # 1 for raising this important point. We have discussed the need to explore for the metastasis when executing the model as well as explain how animal strain may affect the probability of metastasis.

5. The authors need to give guidance on how to incorporate new chemo-preventives or novel immune-modulators, as these are important applications of the DMBA/TPA protocol. Typically, this would include dose responses, and choosing DMBA and TPA doses on the midpoint of the tumor response curve such that and increase or decrease in tumor number brought about by the modifier might be detected.

Authors´ reply and action: We thank reviewer # 1 for raising these important points. Accordingly, we have discussed these issues in the discussion part of the manuscript.

6. This reviewer understands the utility of the DMBA/TPA model for carcinogenesis for studying mechanisms and modifiers of carcinogenesis; however, this model is not exactly relevant to the human condition. The authors should therefore include a paragraph on merits of the DMBA/TPA model over the solar UV radiation model.

Authors´ reply and action: We have discussed both UV radiation model and the general applicability of DMBA-TPA model to human disease in the revised version of the manuscript. Unfortunately, that extended the discussion to seven chapters, i.e. beyond the space limit set by the JoVE.

7. The text is in need of moderate English language editing.

Authors´ reply and action: We have paid special attention to the English language to improve the presentation throughout the text.

8. The title should not include abbreviations.

Authors´ reply: We are aware of the JoVE`s policy of avoiding abbreviations in the title. However, the abbreviation DMBA-TPA is a widely used and recognized term in the field of cancer research.

Authors´ action: We felt that removing the "(DMBA-TPA)" from the title would diminish the informational value of the title substantially. Therefore, we decided to keep the title as it stands.

Reviewer #2:

Manuscript Summary:

This manuscript describes detailed experimental procedures for a standard DMBA/TPA induced mouse skin carcinogenesis study. Stain differences in this carcinogenesis model are also provided. Last, DMBA/TPA-induced cutaneous inflammatory response is presented.

Major Concerns:

No major concerns.

Authors´ reply and action: We thank reviewer # 2 for his/her positive opinion on our manuscript.

Minor Concerns:

Figure 1: TPA applications start in Week 2, not Week 1.

Authors´ reply and action: The Figure 1 has been revised accordingly.