

## Peer Review File

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### Reviewer Comments

#### Introduction

**Comment 1:** There is a spelling mistake on line 15 of page 3 with the word “consistent”.

**Reply 1:** Thanks for pointing out the typo. We have modified our text (see Page 4, line 11).

Changes in the text: We also evaluated the anti-cancer activity of COX-2 inhibitor celecoxib on SCC tumorigenesis in vivo by intraperitoneal injection, and found it exerted very potent cancer inhibitory activity, which is now consistent with data from COX-2 knockout mice.

#### Methods

**Comment 2:** On line 4 of page 4 the 2 in CO<sub>2</sub> should be subscript.

**Reply 2:** Thanks for pointing out the error. The 2 in CO<sub>2</sub> has been changed to a subscript (see Page 4, line 27).

Changes in the text: These cells were cultured in low-calcium DMEM/F12 (3:1) supplemented with 10% fetal bovine serum (FBS, Gibco Co., USA) at 37 °C in a humidified atmosphere of 5% CO<sub>2</sub> and 95% air.

**Comment 3:** Lines 16-18 on page 4 are grammatically awkward. Consider rephrasing.

**Reply 3:** Thanks for your advice. We have divided the sentence into two (see Page 5, lines 13-15).

Changes in the text: A431 or SCC-13 cancer cells infected by lentivirus expressing scrambled or COX-2 shRNA were grown in 6-well plates with duplicate. When reaching a confluence of ~95%, cells were treated with mitomycin C to arrest growth.

**Comment 4:** Spell out the “8” on lines 19 and 21 of page 4. The line “8 areas of scratches lines were marked” is confusingly worded. Consider rephrasing.

**Reply 4:** Thanks for the advice. We have rephrased the sentence and given more details. (see Page 5, lines 16-19).

Changes in the text: Total eight areas down through the two scratched lines were labeled on the bottom side of the plate by marker pen, and immediately recorded

by camera; after 24 hours when the scratches almost closed, the same eight scratch areas were recorded again.

**Comment 5:** Spelling mistake of “xenograft” on page 5, line 15.

**Reply 5:** Thanks for pointing out the typo. We have modified “exnografted” to “xenografted” (see Page 5, line 7).

Changes in the text: When tumor diameter reached 1.5 cm, all of mice were euthanized and xenografted tumors were harvested.

**Comment 6:** Page 5, line 17, the word “of” is not needed.

**Reply 6:** Thanks for your advice. We have deleted the “of” as advised (see Page 5, line 10).

Changes in the text: When tumor reached the endpoint, all mice were euthanized and tumors were dissected, photographed and weighed.

## Results

**Comment 7:** On line 20 of page 6, “significant reduction” is an exaggeration. Whilst the blots of A431 do indicate shCOX-2 successfully reduced expression, the adjective “significant” (line 20, page 6) is an overstatement on the basis of the image provided. The SCC-13 data is more believable, but the blots are still distorted. It would be good for the original images of the blots to be included as the supplementary data – although I leave this decision up to the editors.

**Reply 7:** Thanks for your advice. We have modified “a significant reduction” to “an obvious reduction” as advised (see Page 7, line 7). We used the same shRNA to knock down COX-2 in different cell lines several times (see figure 2a, 3d and 3h) and observed similar results.

Changes in the text: Western blot results demonstrated an obvious reduction of COX-2 expression in cancer cells infected with lentivirus expressing COX-2 shRNA (Fig. 1a).

**Comment 8:** The explanation of the methodology in lines 15-22 of page 8 are repetitive of those provided in the methods.

**Reply 8:** Thanks for your advice. We have removed the following sentences as advised (see Page 8, lines 30; Page 9, lines 1-3): A431 cancer cells were implanted under the skin of nude mice. When tumors size reached ~35 mm<sup>3</sup> after 20 days, the mice were randomly divided into treatment and control groups (all Balb/c female nude mice were 9-week old with similar body weight and housed under SPF condition), and 5 mg/kg COX-2 inhibitor celecoxib or the

same volume of control solvent were administered by intraperitoneally injection everyday, followed by measuring tumor growth twice a week. Changes in the text: To further examine the efficacy of targeting COX-2, we treated nude mice bearing xenografted SCC tumors by intraperitoneally injecting the COX-2 inhibitor celecoxib. As revealed by tumor growth curves (Fig. 5a) or final tumor weights (Fig. 5b and 5c), celecoxib significantly inhibited the tumor growth.

#### Discussion

**Comment 9:** Is a word missing following “inflammatory” on line 27 of page 9?

**Reply 9:** Thanks for your advice. We have modified “inflammatory” to “inflammatory cytokines” as advised (see Page 9, line 5).

Changes in the text: COX-2 is induced by inflammatory cytokines in cancer and further promote inflammation, therefore forming a reciprocal positive feedback to accelerate cancer progression (24, 25).

#### Conclusions

**Comment 10:** “in vitro” and “in vivo” on lines 13-14 of page 10 should be italicised.

**Reply 10:** Thanks for your advice. We have modified our text as advised (see Page 10, lines 19-20).

Changes in the text: COX-2 knockdown potently inhibits proliferation of cancer cells in vivo but not in vitro in SCC, indicating that COX-2 might impact on the interaction between cancer cells and surrounding microenvironments rather than on cancer cells directly.

**Comment 11:** On line 15, page 10 a period should follow the word “directly” rather than a comma.

**Reply 11:** Thanks for pointing out the erro. We have modified our text as advised (see Page 10, line 21).

Changes in the text: COX-2 knockdown potently inhibits proliferation of cancer cells in vivo but not in vitro in SCC, indicating that COX-2 might impact on the interaction between cancer cells and surrounding microenvironments rather than on cancer cells directly.