Author's response to reviews

Title: Genes Associated With Prognosis After Surgery For Malignant Pleural Mesothelioma Promote Tumor Cell Survival In Vitro

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Author's response to reviews:

All changes requested by the reviewer's were made. The reviewer's did not request any new experiments hence no new data is included in the revised manuscript. Specific responses are below.

Response to reviewers:
(Responses in all caps)

Reviewer's report:

Comments on the paper by Gordon et al.

The paper is interesting and highly relevant. Testing candidate target genes discovered by microarray should be of high priority, and a thorough siRNA and microscopy study has been carried out here.

As this must be published, I have some questions and comments that should be clarified.

Authors contribution: Who actually produced the experimental data?
THIS HAS BEEN CLARIFIED ON THE TITLE PAGE.

Introduction:

Chemotherapy has improved survival the last decade, although not impressive, from 9.3-12.1 months.
AGREED.

Materials and methods:

A549 is as far I am concerned a squamous cell lung cancer. (Br J Cancer. 2001 Aug 3;85(3):446-52.

The ability to accumulate deoxyuridine triphosphate and cellular response to thymidylate synthase (TS) inhibition.
Webley SD, Welsh SJ, Jackman AL, Aherne GW.
CRC Centre for Cancer Therapeutics, Institute of Cancer Research, 15 Cotswold Road, Sutton, Surrey, SM2 5NG.)

The origin of the cells should be noted in the methods chapter as well. Since the test was discovered for mesothelioma, why not use a mesothelioma cell line? Are there studies showing that mesothelioma cell lines are not good in high throughput and in transfection studies?

THESE CHANGES HAVE BEEN MADE. WE ARE NOT AWARE OF ANY PUBLISHED STUDIES SHOWING THAT MESOTHELIOMA CELLS ARE NOT IDEAL FOR HIGH THROUGHPUT ANALYSIS. WE HAVE CLARIFIED STATEMENTS TO THIS EFFECT IN THE MANUSCRIPT TO REFLECT THE FACT THAT THIS WAS OUR OWN PERSONAL OBSERVATION.

Discussion:

A positive finding is that downregulation of those genes in the squamous cell line may induce apoptosis, so those genes may be targets for other cancers as well. There was also an effect on the normal cells, so a note on the dangers of harming normal cells could be on its place. A natural continuation of this study is to look at mesothelioma cell lines and or animal models.

AGREE. A COMMENT TO THIS EFFECT HAS BEEN INSERTED IN THE DISCUSSION.

Corrections Page 5, line 12: ARHGDIA instead of ARHGIA Page 11, line 3: A549 are derived from a squamous cell lung tumour. Page 11 line 12: ..a normal cell line, WI 38...

THESE CORRECTIONS WERE MADE.

Fig 5: Why is the cell number index higher in the tumour cells that were knocked down for the genes ARGDIA, COBLL1, TM4SF1

Should be discussed.

A DISCUSSION OF THIS POINT HAS BEEN ADDED. WE DO NOT HAVE ANY DATA SUPPORTING A CAUSE FOR THIS OBSERVATION, BUT HAVE SUGGESTED THAT THE EFFECT IS LIKELY DUE TO THE INCREASED IMPORTANCE OF THE POSITIVE CONTROL GENES (RELATIVE TO THE EXPERIMENTAL GENES) IN THE PROCESSES BEING MODELED.

Reviewer's report:

- Major Compulsory Revisions

This is a technically well-conducted study in a therapeutic area that is in great need of innovation by a major established group in mesothelioma research. The authors have pointed out the major limitation of this study, namely the choice of WI38 and A459 cell lines to model the potential biological role of the 4 genes linked to prognosis in mesothelioma. Of note, hTERT immortalized human mesothelial cells are now available in some centers for study (via Prof Gazdar, Texas). Bearing this in mind and based on the available literature, the following
revisions are indicated.

1) Knockdown of PKM2 mRNA has been shown recently to induce apoptosis in the A549 cell line in vitro and in vivo (Shi et al, Cancer Sci 2010 101(6):1447-53). In view of the use of the same cell line with differing results, this paper should be referenced and discussed in the current manuscript.

THIS HAS BEEN DONE AND DISCUSSION ADDED AS A RESULT.

2) Knockdown of ARHGD1A triggered apoptosis in both the normal and the malignant cell line, thereby questioning the utility of ARHGDIA as a suitable therapeutic target in cancer - especially in view of the renal failure noted in ARHGDIA (-/-) mice. The conclusions are therefore only pertinent to COBLL1 and TM4SF1 as potential tumor specific targets.

THIS LIMITATION HAS BEEN NOTED.

3) The role of surgery, such as EPP, in MPM remains debatable and this should be acknowledged in the manuscript using more circumspect language.

THIS HAS BEEN DONE IN THE INTRODUCTION.

- Minor Essential Revisions
  1) Grammar: paragraph 6 of discussion: "belongs to a family"
CORRECTED

2) The second sentence of paragraph 8 of the discussion "Although Experimentally" is long and needs revising.
REVISED ACCORDINGLY

3) Remove "due primarily to excessive variability" in paragraph 7 of the discussion.
THIS HAS BEEN DONE.