PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	SULF2 expression by immunohistochemistry and overall survival in esophageal cancer
AUTHORS	Lui, Natalie; van Zante, Annemieke; Rosen, Steven; Jablons, David; Lemjabbar-Alaoui, Hassan

VERSION 1 - REVIEW

REVIEWER	Mathewos Tessema Associate Scientist Lovelace Respiratory Research Institute Albuquereque, NM, USA. I have no conflicts of interest
REVIEW RETURNED	18-Jul-2012

THE STUDY	Is the antibody used commercially available? If so describe the source. If not, comparing the utility of commercially available antibodies using a subset of samples will greatly help to assess the reproducibility of these findings and their potential use in clinics. How was the staining percentage calculated (how many cells were evaluated per slide) The intensity score (weak, moderate, and strong) is subjective and hard to replicate in other laboratories/hospitals or by other pathologists. Quantitative assessment of SULF2 expression (protein or transcripts) could help to overcome this issue.
RESULTS & CONCLUSIONS	Staining percentage that was associated with prognosis not staining intensity should be given more attention in the manuscript. Thus, replacing figure-2 with cases that were scored at low, moderate, and high staining percentage, and showing the survival of such case will be more informative than the current figure-2. Some of its discussions such as the importance of detecting SULF2 in blood or other body fluids for diagnosis and prognosis as well as its detection in patients with Barrett's esophagus and severe gastroesophageal reflux to identify high risk cases for esophageal cancer are not supported by any data or literature evidence.
GENERAL COMMENTS	Be consistent in using SULF2 or SULF-2 (page 12) as the gene symbol.

REVIEWER	Caroline Bret INSERM U1040 "Cellules souches normales et cancéreuses" Institut de Recherche en Biothérapie CHRU de Montpellier FRANCE Competing interest: none.
REVIEW RETURNED	31-Jul-2012

GENERAL COMMENTS	1. In the introduction (page 4), the authors should add that, in addition to heparan sulfate chains, HSPGs can bear chondroitin sulfate chains. 2. In the section "Methods", the authors should indicate the signification of the abbreviation "UCSF" (University of Califonrnia San Francisco). 3. In the section "Methods", the authors should precise the drugs used during neoadjuvent therapy and the time between this treatment and the realization of the surgery. 4. In the section "Methods", the authors should not indicate the name of the pathologist. 5. In the table 1 and in the table 2, the authours should replace "race" with "ethnic group" as well as in the text (pages 6, 10).

VERSION 1 – AUTHOR RESPONSE

Reviewer: Mathewos Tessema
Associate Scientist
Lovelace Respiratory Research Institute
Albuquereque, NM, USA.

I have no conflicts of interest

1. Is the antibody used commercially available? If so describe the source. If not, comparing the utility of commercially available antibodies using a subset of samples will greatly help to assess the reproducibility of these findings and their potential use in clinics.

The antibody is commercially available from AbD Serotec (MCA5692T) and Novus Biologicals (NBP1-36727). The Methods section of the manuscript was updated with this information.

2. How was the staining percentage calculated (how many cells were evaluated per slide)?

The pathologist estimated the percentage of tumor cells staining by evaluating all of the tumor cells on the slide. Thus the number of cells evaluated per slide depended on the size of the tumor and varied widely.

3. The intensity score (weak, moderate, and strong) is subjective and hard to replicate in other laboratories/hospitals or by other pathologists. Quantitative assessment of SULF2 expression (protein or transcripts) could help to overcome this issue.

We agree that the intensity score is subjective. We attempted quantitative assessment using Aperio Image Analysis, in particular the Positive Pixel Count Algorithm. However, we found that the results could vary widely depending on the input parameters of the algorithm, and in fact did not correlate well with the pathologist's scores. We decided that since most laboratories and hospitals still use pathologists and not computers to review immunohistochemical stains, that we would analyze the pathologist's intensity score.

4. Staining percentage that was associated with prognosis not staining intensity should be given more attention in the manuscript. Thus, replacing figure-2 with cases that were scored at low, moderate, and high staining percentage, and showing the survival of such case will be more informative than the current figure-2.

Unfortunately, it is difficult to obtain figures illustrating a range of percentage of tumor cells stained, because it is difficult to photograph the entire tumor on the slide in a way that the staining would be obvious. The pathologist used 100X magnification, which required evaluating many fields on each slide.

5. Some of its discussions such as the importance of detecting SULF2 in blood or other body fluids for diagnosis and prognosis as well as its detection in patients with Barrett's esophagus and severe gastroesophageal reflux to identify high risk cases for esophageal cancer are not supported by any data or literature evidence.

We have modified this section of the discussion. We have promising preliminary data in our study on SULF2 in blood, but our project is still ongoing. We meant to highlight only the potential for SULF2, not current evidence.

6. Be consistent in using SULF2 or SULF-2 (page 12) as the gene symbol.

Thank you. We have corrected the gene symbols, so they are all SULF2.

Reviewer: Caroline Bret
INSERM U1040 "Cellules souches normales et cancéreuses"
Institut de Recherche en Biothérapie
CHRU de Montpellier
FRANCE

Competing interest: none.

1. In the introduction (page 4), the authors should add that, in addition to heparan sulfate chains, HSPGs can bear chondroitin sulfate chains.

The Introduction has been modified to include this important fact.

2. In the section "Methods", the authors should indicate the signification of the abbreviation "UCSF" (University of Califonrnia San Francisco).

The Methods section has been modified.

3. In the section "Methods", the authors should precise the drugs used during neoadjuvent therapy and the time between this treatment and the realization of the surgery.

Unfortunately, the drug regimen and time between neoadjuvant therapy and surgery is not known for most of our patients. Since UCSF is a tertiary care center, we operate on many patients who received their initial care at their local medical centers. Details of their previous care was not consistently included in our clinic notes. This is a limitation of our retrospective study.

4. In the section "Methods", the authors should not indicate the name of the pathologist.

The Methods section has been modified.

5. In the table 1 and in the table 2, the authours should replace "race" with "ethnic group" as well as in the text (pages 6, 10).

We understand that our use of race and ethnicity is not technically correct, but it is how the United States government collects its data. For the US Census and other government operations, residents are asked to choose the 'race or races' with which they most closely identify, and the options include both ethnic groups and national origin groups. Residents are also asked to choose the 'ethnicity' with which they most closely identify, either 'Hispanic or Latino' or 'Not Hispanic or Latino'. UCSF uses the same distinction when collecting patient demographics, and thus we included 'race' and 'ethnicity' as variables in this study.

VERSION 2 – REVIEW

REVIEWER	Mathewos Tessema Associate Scientist Lovelace Respiratory Research Institute Albuquereque, NM, USA. I have no conflicts of interest
REVIEW RETURNED	21-Aug-2012

THE STUDY	The method section needs to include how the staining percentages were calculated; responding to the comments alone will not help readers.

REVIEWER	Caroline Bret INSERM U1040 "Cellules souches normales et cancéreuses" Institut de Recherche en Biothérapie CHRU de Montpellier FRANCE
REVIEW RETURNED	09-Sep-2012

- The reviewer completed the checklist but made no further comments.

VERSION 2 – AUTHOR RESPONSE

Comment: The method section needs to include how the staining percentages were calculated; responding to the comments alone will not help readers.

Response: The method section was updated accordingly.