Author’s response to reviews

Title: Potential biomarkers of ductal carcinoma in situ progression

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Author’s response to reviews:
Dear Dr. Gummlich and Dr. Solera,

We thank the reviewers and editors for the constructive feedback on our article. We have revised the manuscript taking those comments into consideration and included a response to the Reviewers’ comments. As commented by Dr. Cohen, we also believe that comprehensive retrospective studies encompassing the 3 genes highlighted in this paper would be of great importance for clinical use of genetics, and we also believe that this transposition into the clinical practice still requires other steps in addition to our contribution. This is a basic research, done as reliably as possible. As suggested by Dr. Schwartz, the cited terms have been fixed, references have been updated, dubious meanings have been clarified and the English language was completely revised.

We appreciate the consideration of this revised submission and look forward to hearing back from you.

Sincerely,
RESPONSE TO THE REVIEWERS

We would like to thank the Reviewers and Editors for their suggestions, which were most helpful in the generation of the final version of this manuscript. A list of responses to each comment/query has been provided below and the manuscript was revised in accordance with those responses. Changes made in the original submission have been highlighted in yellow to facilitate tracking. We appreciate the consideration of this revised submission.

REVIEWER I

Comment 1

This is an interesting and original study trying to find molecular biomarkers which would indicate potential progression to invasive ductal carcinoma and hence suggest the use of more aggressive surgical treatment for a particular DCIS. Many studies using immunohistochemical and molecular markers have been undertaken but as, yet no definite answers have been delineated, including in the present study which only proposes the 3 genes (FGF2, GAS1, SFRP1) as potential markers. They were found in pure DCIS and in the DCIS component with invasive carcinoma. All 6 pure DCIS studied were Grade 3, 3 were comedo carcinoma entirely or as a component, and the 3 genes were unregulated in the pure DCIS vs DCIS component, and in the noninvasive vs invasive carcinoma. Normal breast tissue was used as control.

Many parts of this paper are too long- number of coauthors, Results which are well shown in the Tables 1-3, and the Figs 1-4, Discussion, and References. The 3 genes indicated have not been shown in several other studies referenced, and other references found.
I would really like to see a follow up retrospective clinical study of the 3 genes in DCIS pure and the subsequent invasive ductal carcinoma. Type of treatment (excision vs mastectomy), length of follow up which should be 10-20 years, grade, ER and HER2 status, cost to perform, how long to do the test, and technical skill required are a few parameters which need to be documented in order to assess the use of the gene analysis to manage treatment of DCIS.

Response: The clinical retrospective study, as cited by Dr. Cohen would really be valuable to a better understanding of disease progression and clinical management of DCIS. We believe that the translation of studies such as ours to clinical practice are of fundamental importance, but requires more comprehensive analysis and validation to provide direct and concrete answers. Our study does not intend to bring these markers to a clinic use in this moment, but serve as a guide of potential markers, that would be useful in the future for clinical management. Besides the characteristic of basic research, our study was developed using strict sample selection, involving pathologists and medical oncologists with expertise in clinic and research to select the best cases to guarantee a great representation of each component (DCIS, DCIS component, DCI) and an unambiguous gene expression. Furthermore, the technology used here (Nanostring) has been largely used for FFPE samples which gives more confidence to the data generated. A bioinformatics group from Barretos Cancer Hospital performed the data analysis together with us. Thus, although basic, our study sought confidence in the protocols and analytical steps used in this study, and we believe this data could be useful in the future development of translation studies. A relevant point to consider is that comparison between genetic studies always is biased by sampling and technical variations, so their follow-up and correlation with advancement to histology/clinical/epidemiology will bring us more answers. Our study is the first step in this process.
Comment 1

The goal of this study was to identify potential biomarkers in DCIS that might predict risk of disease progression, i.e., risk of invasiveness. They performed gene expression profiling of six cases each of pure DCIS, pure invasive ductal carcinoma (IDC), and IDC with an associated DCIS component; as well as three cases of non-neoplastic breast tissue. They found that pure DCIS had the most altered gene expression profile; and propose three genes as potential biomarkers of DCIS progression. This is an interesting study with analysis of a lot of data. However, the number of cases studies is small with sweeping conclusions. Furthermore, there is a bias towards more biologically aggressive tumors. Of the pure invasive carcinomas, only 1 of 6 is a luminal A and 3 of 6 are grade 3 tumors. All pure DCIS cases were grade 3. Of the IDC with a DCIS component, no luminal A tumors were included. 4 of the 6 IDC were grade 3 tumors and 4 of the 6 DCIS components were nuclear grade 3 (high grade). This paper might be more appropriate as a much shorter Preliminary Communication.

Specific Comments:

At the bottom of Table 1, it has "IDC-IDC with in situ component". Did the authors mean to have "IDC-DCIS"

Response: This topic has been changed to DCIS-IDC (to match what we use throughout the text) both in the bottom of Table 1 and in Table 1.

Comment 2

Reference 12. Why use the 7th edition of the AJCC Cancer Staging Manual when the 8th edition has been out for 2 years?

Response: The analytical phase, including patient selection in conjunction with the partner clinical oncologists, reagent’s purchase procedures, and protocol definition were performed since June 2015. Sample selection, which considered the TNM clinical stage, was performed based on the seventh edition. Throughout the manuscript writing and bioinformatics analysis, the new edition (eighth) was already in effect and should be used. Given this important observation, we were able to verify and benefit from the news contained in the new edition. Thus, we have included the new edition in our references (now reference 13 – line 434), and also kept the old version, which was used at the time of sample selection.
Comment 3

Reference 13. Why use WHO Classification of Tumors published in 2003 rather than the more recent one published in 2010?

Response: The old reference has been changed by the latest edition about breast tumors (2012 – now reference 14 – line 439) and the information were updated as well.

Comment 4

Materials and Methods. Study population. Why use clinical staging rather than pathologic staging?

Response: The authors agree with this change. The term “clinical stage” was altered to “pathological staging” – line 79. We agree that this term better defines the inclusion criteria.

Comment 5

Figure Captions. figure 2. "... most likely involved in the acquisition of pure DCIS invasive capacity" What does that mean? Do the authors mean "invasive capacity of pure DCIs"?

Response: We mean exactly “. Genes most likely involved in invasive capacity of pure DCIS are marked with an asterisk” – lines 596-597. This sentence has been changed for clarification.

Comment 6

Figure 5. What is "primary tumor"? What is "BC stages"?

Response: In this figure caption, "BC stages" would be the "stages of breast cancer progression" (stages 1, 2 3 and 4 as used on the TCGA project). – information included on line 614; "primary tumor" refers to the tumor at the origin site (breast). According to the definition of TCGA itself, it would be "A term used to describe the original, or first, tumor in the body" – information included on line 613. The meaning of the acronym TCGA has been included in this figure caption - line 615.