Author's response to reviews

Title: Evaluation of BRCA1-related molecular features and microRNAs as prognostic factors for triple negative breast cancers.

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Version: 2
Date: 12 August 2015

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Response to reviewers’ comments.

Reviewer 1.

- The reviewer requested a better reference to “BRCA1 mRNA” and “BRCA1 gene”. In order to make that point clearer, we modified the BRCA1 annotations to allow an easier distinction between the gene BRCA1 gene (italics), the mRNA (BRCA1 mRNA) and the protein (BRCA1 or BRCA1 protein) (cf lines 79, 88, 98, 99).

- The reviewer wondered whether we could make the analyses on two separate cohorts, based on patients age. Indeed, we agree with the reviewer that the biology of the tumors might be different in younger or older patients. However, our cohort contained only five patients below 35 (cf additional file 3), and, in these conditions, we could not observe any significant difference between those two groups. As we consider that any analysis made on such low numbers of patients are questionable, we did not include them in the revised manuscript.
Response to reviewers’ comments.

Reviewer 2.

- The reviewer wrote that “in the introduction section, BRCA1-related parts could be mentioned briefly. Besides, it could be described the relationship between BARD1 and BRCA1 specifically.”

Indeed, it is important to put our work in the perspective of BRCA1 biological function. As suggested, we added in the introduction a list of the BRCA1-related parameters that were measured in this article (cf line 106-108). We wrote: “we quantified molecular parameters focusing on the BRCA1 gene expression regulation and function (BRCA1 promoter methylation, BRCA1 in situ mRNA expression, BRCA1 in situ protein expression and BRCA1 in situ interaction with BARD1) in 69 TNBC. In addition, the BRCA1-BARD1 interaction needed for BRCA1 function was mentioned in lane 61-62. We wrote: “BRCA1 is involved in large protein complexes and its interaction with other proteins, as BARD1, is required for its function.”

- The reviewer considered that “the reasons that why these miRNAs are chosen was not clear in the introduction" Indeed, we preferred to describe the reason why those microRNAs were chosen in the material and method section (cf line 152) and in the Additional Table File 2. This choice had been made to allow, in the introduction, a clear and straightforward description of why new prognosis factors are needed in this clinical field. We think that the description of the miRNA choice, because of its length and complexity, would be confusing if placed in the background section.

- The reviewer indicated why microRNAs were chosen (line 146 of the original manuscript). “However, which one have role in which pathway? Authors may be add a table to show these properties or may be used pathway program.” Indeed, it is of putative interest for the reader to know whether the explored microRNAs have a known function that is relevant for breast cancers.

As suggested by the reviewer, the additional table 2 was completed to contain the MSigDB Canonical Pathways information’s, as provided by the StarBase v2.0 web server issue (1. Li JH, Liu S, Zhou H, Qu LH, Yang JH: StarBase v2.0: Decoding miRNA-ceRNA, miRNA-ncRNA and protein-RNA interaction networks from large-scale CLIP-Seq data. Nucleic Acids Res 2014, 42:92–97). The most significant MSigDB Canonical Pathways affected by each microRNA measured in this article is mentioned. The legend of the additional table 2 was modified in consequence in line 524-527: “Additional Table File 2: List of the microRNAs quantified by RT-qPCR, their sequences and the reasons for their choice for the study. The most significant MSigDB Canonical Pathways affected by each microRNAs are mentioned as provided by the StarBase v2.0 web server issue [39].

- The reviewer questioned why we “did not include adjacent tissue in this study. The study includes comprehensive statistical analysis. However, this data might be added to obtain comparison data.”

The tumoral tissues that were analyzed in this study were obtained from our biobank and came from patients subjected to surgery many years ago in order to get a long term follow-up and allow statistical analyses on new prognostic parameters linked with relapses. Unfortunately, in these
conditions, adjacent healthy tissues were not available, preventing any further analysis. Moreover, we studied prognostic parameters by comparing tumoral samples from TNBC. A comparison of biological parameters between every tumor and the adjacent tissue is another question that does not fall within the scope of the present paper.

- The reviewer referred to publications by “Chang et al. Molecular Cancer (2015) 14:36 and Cascione et al. (2013) who studied association microRNAs with triple negative breast cancer. They mentioned determined microRNAs in abstract section. Authors added these detail in abstract and introduction section”.

Cascione et al. (2013) measured the levels of two microRNAs in TNBC that are also investigated in our study (miR-155, miR-374), and that were associated with overall survival. This reference was already cited in the Additional Table File 2 to justify the reason of the choice of the miRNAs studied in our work. As suggested, we also added this reference in the background section (cf line 97).

In addition, we also added the Chang et al. (2015) reference in the Additional Table File 2. Indeed, as the purpose of this article was different from ours (to define signature to differentiate triple negative tumoral from healthy tissue based on miRNAs expression), we did not add this reference in the background section to avoid any confusion in the definition of the aim of our study.

- The reviewer referred to publications by “M. Boukerroucha (2015) and Okeye et al. (2015) who have showed that “miRNAs such as miR-141-3p, miR-203a, miR-548c-3p, miR-607 and miR-96-5p were appreciably upregulated in TNBC. Authors should be added these references.”

The first reference is one of our own papers (Boukerroucha et al., BMC Cancer. 2015 Mar 26;15:181.). In that paper, we reported the occurrence of glioblastoma in patients carrying a BRCA1 mutation and studied BRCA1 expression in these tumors. However, in that paper, we did not explore miRNA expression neither in the breast or brain tumors. There is thus no reason to mention this paper in the context of miRNA expression in TNBC.

In addition, we were unable to find any reference corresponding to Okeye et al. (2015). We also made a PubMed search on miR-141-3p, miR-203a, miR-548c-3p, miR-607 or miR-96-5p and breast cancer but could not identify the reference mentioned by the reviewer.

- The reviewer asked us to add a reference in the discussion section: Cecener et al. (2015) “BRCA mutations cause reduction in miR-200c expression in triple negative breast cancer”.

This is indeed a relevant reference and we added a comment in the discussion section about the effect of the BRCA1 mutation on the tumoral miRNAs expression together with the requested reference (cf lines 273-276: “The work of Ertuk and Cecener also stated that miRNAs expression can be different in BRCA1 mutated or normal TNBC tumors [31]. However, we could not observe similar effect, probably due to the small number of patients.”)

- Minor revision: we corrected "transcriptional" instead of "transcriptionnal" (line 86 of the revised manuscript).