Author's response to reviews

Title: MiR-190b, the highest up-regulated miRNA in ERalpha-positive compared to ERalpha-negative breast tumors, a new biomarker in breast cancers?

Authors:

geraldine g cizeron-clairac (geraldine.clairac@gmail.com)
francois f lallemand (francois.lallemand@curie.fr)
sophie s vacher (sophie.vacher@curie.fr)
rosette r lidereau (rosette.lidereau@curie.fr)
ivan i bieche (ivan.bieche@curie.fr)
celine c callens (celine.callens@curie.fr)

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Author's response to reviews: see over
To: Roselyn Remoto  
BMC Cancer  

RE: BMC Cancer – Revised version of Manuscript 1984564149146023  

Dear Ms Remoto  

Please find herewith the revised version of Manuscript 1984564149146023 entitled “MiR-190b, the highest up-regulated miRNA in ERalpha-positive compared to ERalpha-negative breast tumors, a new biomarker in breast cancers?”.  

We have amended the manuscript according to the specific comments of the expert reviewer(s), as detailed in “Author Response to Reviewer(s)’ Comments”. The changes to the manuscript are also tracked within the document by highlighting modifications in yellow.  

Hoping that the revised version will meet with your approval, we thank you for your kind attention.  

Yours sincerely,  

Dr. Céline Callens,  
Institut Curie – Service de Génétique – Unité de Pharmacogénomique  
26 rue d’Ulm, F-75005, Paris; France,  
Phone: (33) 1 72 38 93 65; Fax: (33) 1 53 10 26 65  
E-mail: celine.callens@curie.fr
Reviewer(s)' Comments to Author:
Reviewer: 1

Reviewer's report
Title: MiR-190b, the highest up-regulated miRNA in ERalpha-positive compared to ERalpha-negative breast tumors, a new biomarker in breast cancers?
Version: 3
Date: 12 January 2015
Reviewer: Patricia de Cremoux

Reviewer's report:
The paper aimed to analyse the miRNA expression in large series of ER-positive and ER-negative-breast cancers, and to determine in this context their functions. This subject is very interesting regarding the international literature on miRNA in breast cancers. Previous published papers with different aims are heterogenous, and no clear conclusions rose from these data's. The method used is adapted, and the team has a large expertise in this domain.

This represents an article whose findings are important to those with closely related research interests, which needs some revisions to be published

1/ Major Compulsory Revisions

1-1/ It is difficult to evaluate prognosis factors without the knowledge of the treatment administered. Consequently, the treatment of patients is missing in the paper. As MFS is one of the main objective, the treatment administrated to patient represents a major criteria that will influence the prognosis. In addition the period of patient's inclusion also will determine the treatment regimen. The samples are well annotated. So the type of chemotherapy and hormone therapy should be very interesting to evaluate.

We thank the reviewer for this pertinent comment.

We added informations concerning the treatments administered in the table 1. In this cohort, none of the ERBB2+ patients was treated with anti-ERBB2 therapy. We compared metastasis free survival (MFS) of patients who have been treated by hormonotherapy alone according to their miR-190b expression level (high or low). There is no difference between MFS of patients treated by hormone therapy alone (p=0.40). We could not realize this analysis for patients receiving other adjuvant treatment because all of them expressed miR-190b at low level.

We added a new paragraph in our manuscript as follows :

“If we compared MFS according to the type of treatment, we observed no prognostic impact related on miR-190b expression level for patients who received hormone therapy alone (p=0.40, data not shown). All patients receiving other adjuvant treatment expressed miR-190b at low level. ”
1-2/ In the context of ER-positive tumors, the response to therapy and the duration of response remains a clear problem. Should the authors also evaluate the RFS or EFS?

We have evaluated EFS according to the miR-190b expression level and we obtained a more significant result than observed in MFS analysis. We changed the paragraph as follows:

“Interestingly high expression of miR-190b was associated with a prolonged metastasis-free survival independently to ER status and treatment (log rank test: p=0.0173, HR=1.869, 95% CI=1.12 to 3.13) (Figure 2A), as well as a prolonged event-free survival (log rank test: p=0.0046, HR=2.048, 95% CI=1.248 to 3.360) (Figure 2B)”.

Page 15 lines 1-4

1-3/ How were selected the first 31 patients and the validation population among the global cohort of patients? Table 1 showed a clear heterogeneity in the characteristics and the events in the screening population and the validation population. This has to be discussed.

We totally agree with the reviewer. All samples analyzed were chosen among primary breast tumors operated at the Centre Rene Huguenin from 1984 to 2009. The 31 primary breast tumors in the screening set included more grade III tumors and more patients <65 years than validation set with the aim to emphasize robustness of genes differentially expressed. The 153 primary breast tumors in the validation set has standard clinical, pathological and biological factors similar than in the whole of breast tumors operated at the Centre Rene Huguenin from 1984 to 2009. Consequently this validation set is a classical representative cohort of breast cancers.

We added a new sentence in the material and methods section as follows :

“In these screening set, we voluntary included more SBR grade III tumors with the aim to facilitate identification of robust genes differentially expressed whereas the validation set is totally representative of breast cancers treated in the Curie institute/René Huguenin hospital between 1984 and 2009.”

Page 6 lines 16-19

1-4 the paper included numerous datas, screening and validation datas, screening of previously published datas and a focus on MiR-190b. In addition descriptive datas and functional datas are presented on human breast cancer cell lines. Perhaps the paper would be more explicit if it was focused only on human breast cancers. The functional datas on human breast cancer cell lines
did not clearly improve the paper (supplemental datas?). Presenting the paper with this aim would improve the lecture and understanding

We followed this suggestion and have moved the two paragraphs concerning functional datas at the end of the manuscript. All figures related to these results are now presented in additional files.

We have made some minor changes in these two paragraphs as follows :

“Effect of estrogen on miR-190b expression
To identify if estrogen could explain the deregulation of miR-190b between ER+ and ER- breast tumors, we measured its expression levels on the ERα-positive MCF-7 and T-47D breast cancer cell lines treated with 17β-estradiol (E2). We did not observe an increase of miR-190b expression levels in MCF-7 or in T-47D treated by E2 whereas the expression of the well-known ERα-induced gene pS2 was highly increased in the two cell lines (Additional files 6 : Figure S3A and S3B). Others 19 miRNAs did not respond to 17β-estradiol either (data not shown).

Role of miR-190b expression in tumor proliferation
The heightened increase of miR-190b in ER+ breast cancer prompted us to explore this possible biological significance in cell proliferation. As initial step, the capacity of proliferation induction was evaluated on breast cancer cell lines ER+ MCF-7 and T-47D that were transfected with an antagonir against miR-190b and on ER- MD-MBA-231 that was transfected with a miR-190b mimic. The efficacy of transfection was verified by quantifying miR-190b in RNA extracted from transfected cells by qRT-PCR (datas not shown). Antagomir did not affect proliferation of MCF-7 (Additional file 7 : Figure S4A) and T-47D cell lines (Additional file 7 : Figure S4B) as miR-190b mimic has no effect on MDA-MB-231 proliferation (Additional file 7 : Figure S4C). Other experiments are therefore needed to decipher the role of miR-190b in mammary tumorigenesis”.

Page 15 Lines 7-26 to page 16 lines1-2

2/ Minor Essential Revisions
2-1/How was evaluated the normal tissue that represents the reference for the expression of MiRNAs ? Reduction mammectomy and adjacent tissue from breast cancer patients do not represent the same tissue. In the mat and methods chap, 12 normal samples were presented, then, 8 in the screening population and 8 in the validation population. Could you clarify this point?

Normal breast tissue samples were checked histologically and selected on the basis of their cellular composition. Samples with a majority of stroma, inflammatory cells or mast cells were excluded from the study, the proportion of epithelial cells was more than 60% in all the normal breast tissue samples.

We only have twelve specimens of normal breast tissue and due to the rarity of such samples, we used 8 among the 12 as control for the screening set and 8 among the 12 as control for the validation set (4 of them are shared with controls used for the screening set).

We did not find expression difference of miRNA between adjacent tumor derived- and reduction mammoplasty derived- normal tissues. Moreover, Finak et al. (2006) found
that the molecular signatures that distinguished breast reduction tissue from tumor-
adjacent normal tissue were absent. Subsequently these authors concluded that
morphologically normal tissue adjacent to breast carcinomas has not undergone
significant gene expression changes when compared to breast reduction tissue, and
provide an important gene expression dataset for comparative studies of tumor
expression profiles (Finak, 2006).
We added this reference to the control samples section of our manuscript as follows:

“Control samples consisted of twelve specimens of normal breast tissue obtained
from women undergoing cosmetic breast surgery or adjacent normal breast tissue
from breast cancer patients [21].”

Page 6 lines 8-10


2-2/ Recent publications that were published during submission might be
added

We added several recent bibliography references in the introduction and discussion
sections:

Yahya SM, Elsayed GH: A summary for molecular regulations of miRNAs in

Graveel CR, Calderone HM, Westerhuis JJ, Winn ME, Sempere LF: Critical
analysis of the potential for microRNA biomarkers in breast cancer management.

Kaboli PJ, Rahmat A, Ismail P, Ling KH: MicroRNA-based therapy and breast
cancer: a comprehensive review of novel therapeutic strategies from diagnosis to

Aakula A, Leivonen SK, Hintsanen P, Aittokallio T, Ceder Y, Borresen-Dale AL,
Perala M, Ostling P, Kallioniemi O: MicroRNA-135b regulates ERalpha, AR and
HIF1AN and affects breast and prostate cancer cell growth. Mol Oncol 2015.

Li D, Xia H, Li ZY, Hua L, Li L: Identification of Novel Breast Cancer Subtype-
Specific Biomarkers by Integrating Genomics Analysis of DNA Copy Number
Aberrations and miRNA-mRNA Dual Expression Profiling. Biomed Res Int 2015,
2015:746970.

We modified the section discussion as follows:

“We could note that we did not select miR-135b because of absence of expression in
our screening series whereas this microRNA would be differentially expressed in
ER+ and ER- breast tumors, and is described by Aakula et al. as a regulator of
ER[37]”
The article needs some language corrections before being published

The manuscript has been checked for English usage.

Reviewer: 2

Reviewer's report
Title:
MiR-190b, the highest up-regulated miRNA in ERalpha-positive compared to ERalpha-negative breast tumors, a new biomarker in breast cancers?
Version: 3
Date: 27 March 2015
Reviewer: Ratna Vadlamudi

Reviewer's report:
In this manuscript, authors investigated miRNAs that are differentially expressed in estrogen receptor positive (ER+) and negative (ER-) breast cancer using a large cohort of (106 ER+ve and 78 ER-) primary breast tumors. Author’s results identified 20 miRNAs to be significantly deregulated in ER+ compared to ER- breast cancers (12 up-regulated and 8 down-regulated miRNAs). The expression of these altered miRNA were validated using 30 different breast cancer model cells and found upregulation of five miRNAs in ER+ve models as observed in breast tumors. Further, they identified miR190 as a miRNA that is uniquely upregulated in ER+ve cells and its expression is lost in ER-ve breast cancer. Further, miR190b status correlated well with metastasis free survival irrespective of ER status. Collectively these results suggest that miR190 may represent a novel biomarker of metastasis-free survival. However, the mechanistic studies are weak. Overall this is a good study, experiments in general are well designed and conclusions are supported by the data and findings are of interest to the readers of BMC cancer.

Minor essential revisions:
1. Fig 3: It remain unknown whether the lack of effect on the model cells by miR190b- antagonim or -mimics could be due to poor functionality of the reagents used. Additional data in support of the functionality of the reagents used to target miR190 is needed.

We totally agree with the reviewer. We bought commercial miR190b mimic (reference MSY0004929 by Qiagen) and inhibitor (reference MIN0004929 by Qiagen) whom performances have been validated by the manufacturer. Moreover we verified effectiveness of our transfection experiment and specificity for mir190b by quantifying mir190b in RNA extracted from transfected cells. Quantitative RT-PCR results confirm effectiveness of mimic and antagonim to modulate mir190b level (data not shown).

We added a new sentence in our manuscript as follows:
“The efficacy of transfection was verified by quantifying miR-190b in RNA extracted from transfected cells by qRT-PCR (datas not shown).”

Page 15 Lines 22-23

2. The mechanistic studies linking E2 signaling to miR190b are weak

This is a pertinent remark. We have evaluated the effect of E2 on miR-190b and we did not observe changes as illustrated by the figure 2. In parallel, we have also tested the effect of tamoxifen, an antagonist of estrogen receptor, on miR-190b expression in MCF7 cells but this experiment does not have interest because E2 has no effect on miR-190b expression. So we decided to skip this result. As written in the discussion section, we think that miR-190b could be regulated by ER-signaling pathway independent of E2 but we do not have demonstrated it in this paper.

We added a new sentence in the result section as follows:

“Obviously we neither observed effect of tamoxifen treatment on miR-190b expression in MCF-7 cell lines (datas not shown).”

Page 15 Line 14-15

3. Stats need to be included for Fig1 and Fig 3

We apologized for this mistake. Stats are now included in figures 1 and S4 (ex fig 3).

4. Discussion should include potential target genes of miR190b and their role in Metastasis

As suggested by the reviewer, we have more discussed the previous studies concerning miR-190b as follows:

“Few studies reported miR-190b implication in cancers. A recent next generation sequencing project identified mir-190b among 7 others microRNAs as a biomarker for the diagnosis of Merkel cell carcinoma[38]. In lung cancer, miR-190b could be detected easily in serum of patients to facilitate diagnosis[39]. MicroRNA expression profile associated with response to neoadjuvant chemoradiotherapy in locally advanced rectal cancer patients included miR-190b[40]. However none of these studies explored mechanism of action of miR-190b and its targets did not have been well described contrary to miR-190 that could interfere with VEGF-mediated angiogenesis[41]. Moreover miR-190b has not been selected by previous microarray breast cancer studies [16, 17, 19, 42] so we tried to decipher its properties in breast cancer.”

Page 17, lines 21-26 to page 18 lines 1-4

We also added corresponding references:

