Reviewer's report

Title: Androgen receptor expression predicts survival in advanced breast cancer: the role of genetic and epigenetic events

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Reviewer: Marcella Mottolese

Reviewer's report:

The manuscript entitled "Androgen receptor expression predicts survival in advanced breast cancer: the role of genetic and epigenetic events by Peters et al. aimed to determine the potential prognostic role of androgen receptor (AR) in human breast cancer, concomitantly evaluating the regulatory mechanisms that could be involved in the loss of the receptor both in vitro and in human breast cancer tissues. First, the authors analyzed the AR immunohistochemical (IHC) expression preparing TMAs from a small series of 73 human breast carcinomas in order to correlate AR expression with patient overall survival. Then, they studied the DNA methylation of the AR promoter both in breast cancer cell lines and in human breast carcinomas obtained discordant results. The study draws the attention on an interesting and highly debated issue extensively studied so far. In fact, the role of AR as a prognostic marker has been suggested by other authors, though there is a high variability in results obtained. The experimental part of the study, performed on breast cancer cell lines, is complete and well conducted. It is very important to study the regulatory mechanisms underlying the loss of AR to better understand the role of this receptor in breast cancer progression and response to different therapies.

Nevertheless, several additional points need to be addressed mainly in the part of the study concerning human breast carcinomas.

Major Compulsory Revisions

1) Clinical samples (Methods page 10 and Results and Discussion page 5)
This, in my opinion, is the weakest part of the study. Authors assessed, also in the title of the article, that they tested AR expression in a series of 73 advanced breast cancer. They defined advanced breast cancer as grade 3 tumors with positive axillary nodes. This definition should be better clarified. Authors should indicate whether the analyzed tumors are locally advanced breast cancer or advanced breast cancer. The first group of patients encompass a wide range of clinical scenarios including advanced primary tumors (T3/ T4 tumor size), advanced nodal disease (fixed axillary nodes or involvement of ipsilateral supraclavicular, infraclavicular, or internal mammary nodes), and inflammatory carcinomas. On the other hand, women with advanced breast cancer present distant metastases at onset of disease, independent of nodal status. In any case, a high grade tumor is not suggestive of advanced breast cancer. Therefore, authors should better summarize the pathological features of their series of
patients in a separate Table (Table 1 presented in the supplementary data lacks the title and explicative notes. In this form it appears very confused). They should indicate tumor size and presence or absence of distant metastases, the hormonal receptor status, the extent of proliferation index and the HER2 status.

2) The survival data presented in the study is based on AR IHC analysis of TMAs containing two tissue cores of primary cancer. While this sampling is generally appropriate, it does not sufficiently address tumoral heterogeneity. In addition it is unclear whether the authors re-evaluated conventional prognostic factors, as estrogen, progesterone receptors and HER2, on the TMAs. Moreover, is SISH, when necessary, performed on TMAs or using the whole sections? Did the authors consider re-staining the whole slides in discrepant cases? Please discuss the limitations of using TMA in this type of projects and how you address them.

3) It has been reported by several authors that the possible role of the AR as a marker of prognosis is particularly evident among patients with estrogen receptor positive breast cancer, but are rarely present in HER2 positive tumors whereas AR expression has been reported in about 45% of triple negative breast cancers. Taking into account these issues and these variabilities in AR expression among the different breast cancer subtypes, authors should better describe the characteristics of the patients included in the study.

4) Why did the authors correlate AR expression only to overall survival? Is AR expression also correlated to disease free survival? In addition, authors should indicate the median follow up with the minimum and maximum range of survival. To validate a novel biomarker in a complex and heterogeneous disease as it is breast cancer, authors should enlarge their series of patients and should perform more appropriate statistical analyses.

5) In this context, the title appears to be inappropriate and should be changed focusing on the results obtained in cell lines which appear more interesting and more solid than in vivo data.

Minor Essential Revisions

Breast cancer Lines (Methods page10): authors should provide more information concerning breast cancer cell lines used throughout the study (i.e. hormonal receptor and HER2 status).

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests