Reviewer's report

Title: Neuropilin-2 expression in breast cancer: correlation with lymph node metastasis, poor prognosis, and regulation of CXCR4 expression

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Reviewer: paola manduca

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The authors investigate by immunodetection the expression of the Nrp-2 receptor for VEGF in a collection of infiltrating breast cancer tumors surgically operated with removal of lymph nodes. These tumors were previously characterized for the expression of estrogen and progesteron receptors, histological grade, VEGF-C and CXCR4 and lymph node metastasis and the ensuing data have been object of previous reports, and statistical analysis by the same group of research.

In the present report the questions asked are
1- What is the frequency of Nrp-2 expressing tumors?
2- Is there a statistic relationship between Nrp-2 expression in tumors and any of the markers previously detected in the same tumors?
3- Is there a correlation between lymph node metastasis, the expression of Nrp-2 and its co-expression with each or all any of the markers previously detected in the same tumors?
4- Can Nrp-2 expression be considered a prognostic marker?
5- Does inhibition by antibody to Nrp-2 in vitro in the MDA-MB231 line, affect the expression of cytoplasmic CXCR4 and the migration of the cells to CXCL-12?

The authors refer and take a good amount of the data presented in the tables from previous reports of the same group in order to establish the correlations between Nrp-2 protein expression and other markers.

The methods utilized by for the tumor screening by immunodetection are appropriate.

The methods utilized for the in vitro experiments are also appropriate and the controls are included.

The statistical elaborations of the data are appropriate.

By statistic determination, the co-expression of Nrp-2 and CXCR4 is not very highly significantly associated to lymph node metastasis in vivo, while by inhibition studies is shown that Nrp-2 is required for the MDA MB231 cell line migration to CXCL12 in vitro, and that inhibition of the Nrp-2 receptor inhibits CXCR4 cytoplasmic expression in vitro. Although the authors treat the data in vivo and in vitro disjoint, implications are suggested to the reader.
Nonetheless, they do not ask, and do not answer, a relevant question arising from their studies, an the one that could justify the presentation of the data in vivo with those in vitro in the context of the same report, namely if there is co-expression in vivo in the same cell or same region of the tumor of Nrp-2 and CXCR4.

Discretionary revisions—none

Minor essential revision
1-Table 1, Lane 4, 4th column, a bracket is missing, please add.
2-How many sections were utilized to screen for Nrp-2 expression in order to be sure that negatives are such indeed? It is necessary to add this information and information on the positioning within the tumor mass of the sections examined (e.g. marginal/central; adjacent to VEGF-C positive/not).

Major compulsory revision-
1-Co-localized expression of CXCR4 and Nrp-2 in vivo.
There is no proof here provided if Nrp-2 and CXCR4 are expressed in the same positions in the tumor. This should be approached by immunohistochemistry with antibody for each protein on sequential sections of the tumors and must be provided before publication. The two proteins under study, and for which a mechanistical interaction is implied by the presentation of the in vitro data, are both cytoplasmic-transmembrane receptors, both expressed in a variable portion of the tumors cells. The issue of their co-localization in the tumor is accessible for these authors, and the proof is necessary in the context of the data presented in the manuscript.

2- There is a point that should be taken in account in discussing the in vitro results, namely the fact that it is known that expression of CXCR4 in MDA MB231 is dependent from VEGF signaling and it is questionable if the antibody to Nrp-2 is affecting CXCR4 expression directly or through the inhibition of this signaling. The timing of the experiments in vitro is compatible with this kind of secondary event.

**Level of interest:** An article of importance in its field

**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