Reviewer's report

Title: Cross-linking of CD24 inhibits growth of MCF-7 breast cancer cells

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Reviewer: Glen Kristiansen

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The manuscript under consideration describes the effects of CD24 crosslinking using a polyclonal rabbit antibody on various viability parameters in two widely used breast cancer cell lines. After antibody incubation, the CD24 positive cell line MCF-7 displayed a reduced overall viability, a reduced proliferation, a minor growth inhibition in a 3D-cell culture model, increased apoptosis and a reduced number of migrating cells. The authors conclude, that “the blockage of CD24 can be proposed as novel therapeutic strategy for breast cancer treatment”.

Major points.

The work is basically of great interest, since the effects of CD24 crosslinking on breast cancer cell lines are only insufficiently characterized. However, the impact of the presented results would be enhanced, if more than one CD24-positive cell line was analysed. I suggest to analyse at least another CD24 positive breast cancer cell line with the assays described to confound the findings. Also, the choice of the blocking antibody (rabbit polyclonal) should be explained, given the wide range of available monoclonal CD24 antibodies (which might be more suitable for a therapeutic in vivo application). Please describe antibody specificity and the epitope detected by the antibody. This is important, since CD24 is heavily glycosylated.

Please explain the choice of cell lines. MCF-7 is generally considered a model for a less aggressive breast cancer, whereas MDA-MB231 is considered highly invasive. Would it be possible that the suggested anti-CD24 therapy would in vivo target only less aggressive tumour cells but spare more aggressive populations?

The manuscript has a few references that have been cited either inappropriately or in a biased fashion. These phrases should be carefully re-written:

Background, second paragraph, last sentence. Please check the validity of reference 2, because it does not deal with functional properties of rat carcinoma cell lines.

Discussion, line 1. “CD24 plays important roles in progression, migration, and metastasis of human breast cancer”. For this matter of factly sounding statement, references should be cited. However, the role of CD24 in breast cancer is quite unclear. Apart from merely correlative studies that demonstrated a prognostic value of CD24 in primary breast cancer and functional studies, that demonstrated
CD24 as an alternative ligand to P-selectin in breast cancer cells, functional data on the role of CD24 in "progression, migration and metastasis" of breast cancer are scarce. Conversely, different groups have suggested that aggressive breast cancer cells/cases are CD24 negative/low (please see e.g. Breast Cancer Res. 2006;8(5):R59, Proc Natl Acad Sci U S A. 2003 Apr 1;100(7):3983-8., N Engl J Med. 2007 Jan 18;356(3):217-26.), which could be added to the discussion.

Discussion, second sentence. The statement that adhesion was inhibited by CD24 crosslinking is not supported by the authors data ("adhesion (…) was reduced a little, but not significantly", results, last paragraph) and should be omitted.

Discussion, line 19 – “consistent with previous studies showing that polyclonal immunoglobulin inhibits growth of cancer cells” should be further specified since the polyclonal immunoglobulins in the cited study were directed against tumor vasculature.

Discussion, second paragraph –“Using in vitro migrations assays(matrigel) and in vivo immunohistochemical staining, Senner et al. (6) showed that CD24 induces migration of glioblastomas, (…).”, is a simplification. Correct is, that Senner et al. have found in a rat model CD24-positive gliomas more aggressive than CD24 negative implants, but did not observe a greater migration rate of CD24-positive cells in matrigel assays.

Discussion, 4th paragraph –“Furthermore, CD24 is expressed in most neuroendocrine carcinomas of the skin and can thus be applied as a diagnostic marker as well (17). Since CD24 is widely expressed in human neoplasms, it cannot be recommended as an entity-specific diagnostic marker!

Minor points:
Careful proofreading is necessary to eliminate typos.

The rate of CD24 positivity in the abstract (2% and 65%) is different to the results shown later (2% and 61%), please check.

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article of importance in its field

Quality of written English: Needs some language corrections before being published

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.