Author's response to reviews

Title: DMBT1 expression is down-regulated in breast cancer

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Author's response to reviews:

Answer to reviewer's reports
Reviewer 1 (Fernando Schmitt)

Major revisions

1) The reviewer considers that the conclusion that the "down-regulation (?) in carcinomas is consistent with its role in breast carcinogenesis" is an overstatement. We agree to that and changed the corresponding sentence in the abstract to "... its down-regulation in carcinomas suggests a potential role in breast cancer". Whether a down-regulation of DMBT1 takes place in breast cancer and other cancer types is a matter of the perspective. There exists a number of studies that demonstrate that DMBT1 is first upregulated in tumors (except for squamous cell carcinomas), and that this upregulation takes place early during tumorigenesis - as early as the original impact happens (e.g. Mollenhauer et al., 2004). However, at later stages a downregulation may take place, which appears to apply to numerous cancer types including melanomas and lung carcinomas. In a recent study by Sasaki et al. (Histopathology 43: 340-346; 2003) it was shown that upregulation first takes place in liver cancer, but that downregulation of DMBT1 is significantly associated with progression to more invasive stages. Bhattacharjee and co-workers (PNAS 98: 13790-13795; 2001) identified DMBT1 as one of the genes, whose downregulation is associated with worse patient survival. In a manuscript presently under review at a distinct journal, we demonstrate that DMBT1 knockout mice show increased susceptibility to different cancer-causing agents. Taken together, we favor a continuous model for DMBT1 expression and function in cancer, i.e. upregulation at early stages as part of a protective response and downregulation later on during tumor progression.

2) The reviewer asks to quantify Ki 67 in all lesions and study the relation with DMBT1 expression. These are integrated into the revised manuscript. In Immunohistochemistry sections of methods we describe that DMBT1 and Ki 67 were tested in all samples (page 6 line 9). In the results we explain that DMBT1 in cancerous lesion was negative in all but 3 of them (55), and Ki 67 immuno-positivity was quantified as part of the protocol for the surgical pathology diagnosis of breast cancer (Results page 10 line 8,9). DMBT1 expression at protein and at mRNA level did not correlate with histological parameters, hormonal receptors status, proliferation index Ki 67, or other prognostic parameters (Results page 10 lane 11,12 and 19,20). Double staining with anti MCM5 and DMBT1 Abs was performed in 17 benign lesions and a subset of 19 carcinomas where residual normal breast tissue was well represented. We added a figure showing As suggested by the reviewer we have performed three single incubations with anti DMBT1, MCM5 and Ki 67 Abs to compare the respective immunoreactivity (Methods page 6 line 12,13). We added Figure 3 (a,b,c) that show this comparative staining (Figure 3 of the original manuscript is now figure 4). We have added a comparative quantification of MCM5 and Ki 67 immunopositivity in normal breast epithelium (Results page 10 lane 4-7).

3) The referee considers it inappropriate to hypothesize that SP-A and DMBT1 co-expression serves as protective barrier and feels that double immuno staining experiments would be required. We hypothesized that SP-A and DMBT1 co-expression is part of a protective response. This hypothesis is qualitatively


different from serving as a protective barrier. Double immuno staining experiments are not necessary. Even if both proteins would be expressed by different cells, both are secreted luminally, so that they will get in contact in the extracellular compartment.

Minor revisions
1) We have modified table 2 in order to render it more clear and readable
2) We have added in the results section the immunopositivity scores of cancerous lesions (page 9 lane 10,11,13,14) and other statements already mentioned in the major revisions.
3) According to reviewer suggestion we have changed the description of instrumentation: automated slides stainer (Biogenex, S.Ramon, CA, U.S.A.) and reagents (Applied Biosystems, Foster City, CA, USA)
4) We have corrected the spelling of gene and protein product.
5) We have modified sentence at page 11 now line 18.
6) We have corrected typos and grammatical errors.

Answer to reviewer's reports
Reviewer Ndegrees2 (Jaqueline Shaw)

Major revisions

1) The major concern of Prof. Jacqueline Shaw is that we do not consider reasons for downregulation of DMBT1 expression and that this should be included in the discussion. We integrated a corresponding paragraph.

Minor revisions
1) We have corrected the discrepancy between ICH positivity score in methods and in table 2. Now the ICH positivity score in methods (page 7 line 2-4) and in table 2 are consistent.
2) The reviewer asks for more clear presentation of ICC double immunostaining. Double staining with anti MCM5 and DMBT1 Abs was performed in 17 benign lesions and a subset of 19 carcinomas where residual normal breast tissue was well represented (Methods page 6 line 9-12) The results of MCM5 immunopositivity, compared with that of Ki 67, are referred only to normal breast epithelium because all proliferative benign or cancerous lesions were diffusely decorated with anti MCM5 Ab (page 10 line 3-8)
3) The reviewer notes that Ki 67 data are not complete. On sections from same samples used for double staining now we performed also three single incubations with anti DMBT1, MCM5 and Ki 67 Abs to compare the respective immunoreactivity (Methods page 6 lane 12,13 and Figure 3 a,b,c) A comparative quantification of MCM5 and Ki 67 immunopositivity in normal breast epithelium was performed (Results page 10 line 4-7). The evaluation of Ki 67 immunoreactivity in cancerous lesions was tested in all samples and quantified for the surgical pathology diagnosis of breast neoplasia together with hormonal receptors status and other prognostic parameters.