Author's response to reviews

Title: Expression of the embryonic stem cell marker SOX2 in early-stage breast carcinoma

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Expression of the embryonic stem cell marker SOX2 in early-stage breast carcinoma
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We thank the referees for their very helpful comments which have improved the quality of our manuscript.

To address the reviewers’ comments we have performed additional experiments and modified the manuscript accordingly. The changes in the manuscript are highlighted in grey to enable faster processing. Below we address in detail point-by-point the reviewers’ questions and comments.

Reviewer 1:

Major Compulsory Revisions:

We have revised the presentation of the qRT-PCR data as requested and show now an independent panel for each gene (see revised Figure 3). The analysis has been performed in triplicates. Statistic analysis has been performed using the TTEST and added to the figure. Figure 3 is now referred to on page 10 line 7 of the revised manuscript.

The fresh frozen samples have been collected prospectively from patients diagnosed of breast cancer at the University of Tuebingen Institute for Pathology in the years 2009 and 2010.

Minor Essential Revisions:

We have corrected the error in the references section.

Discretionary Revisions:

We have changed “Wilbertz et al, submitted” to “Wilbertz & Perner, personal communication”(see page 9, last paragraph, of the revised manuscript). We actively collaborate with Theres Wilbertz and the research group of Professor Perner.

Reviewer 2:

To 1: We have examined the expression of the SOX interacting gene ALX4 in the samples where material was still available and find that samples expressing SOX2 indeed also express ALX4. The data are now presented in Supplemental Figure 1.

To 2: Data collected from the SAGE database confirms co-expression of SOX2 gene with other embryonic genes as well as SOX2OT and the interacting genes ALX4 and PAX6 in breast (cancer)
cellular (sub)-populations (see below). For the current study, we have mainly focused on SOX2 analysis on protein level. We thank the reviewer for this helpful suggestion that will also guide us during future studies.

To 3: We have performed SOX2OT analysis by qPCR in the samples of which material was still available and present the data in Supplemental Figure 1 of the revised manuscript. SOX2OT was expressed in all samples previously shown to express SOX2 and not detectable in sample 7 where SOX2 expression was also not consistently detectable. As measured by qPCR, the levels of SOX2 OT expression correlate with SOX2 expression levels in some cases (samples 1, 2, 3, 9, 12 and 14) while in some SOX2OT expression was higher than would have been predicted by SOX2 levels (samples 16, 17, 18 and 19). We have inserted the data on page 10 in the Results section and the corresponding methods description on page 8 of the revised manuscript.

**Minor editorial suggestions:**

We have inserted the comma.

**Reviewer 3:**

**Major comments:**

- The manuscript text (see Materials and methods section, page 5 of the revised manuscript) has been modified to more clearly introduce the analyzed patient cohort.

- The FISH analysis has been further extended and performed now on eleven SOX2 positive samples (7 belonging to expression score 3 and 4 of score 2), 4 SOX2 negative samples as well as 3 lymph-node samples showing high SOX2 expression (score 3). Only one primary tumor (with score 3 SOX2 expression) showed a low level SOX2 amplification by FISH, while in all the rest, including the lymph nodes with high SOX2 expression levels, no amplification
events were detectable. The information has been added to the Results section on pages 9-10 of the revised manuscript.

- The samples used for RNA analysis were collected after the ones included in the larger retrospective study on protein level. No fresh frozen material was available from the retrospective study.

- As requested, we have performed a statistical analysis of conventional histopathological parameters and lymphonodal status in our cohort. We found that indeed larger tumor size (T>1) was significantly associated with positive lymphonodal status: 15.5% (9 out of 58) T1 tumors showed lymph node metastases and 51.9% (14 out of 27) T2 tumors (p=0.001; Fishers´s Exact Test). With respect to grading a trend to more frequent positive lymphonodal status was documented in G2/3 tumors (30.9%; 21 out of 68 tumors) in comparison to G1 tumors (11.8% lymphonodal positive tumors; 2 out of 17 analyzed tumors) but was not statistically significant (p=0.138; Fisher´s Exact test).

Minor comments:

Luminal A tumors were defined as tumors with expression of one or both hormone receptors without overexpression of Her2. Luminal B tumors express one or both hormone receptors and show also Her2 overexpression. The information has been added to Table 2 in the revised version of the manuscript.