Author's response to reviews

Title: SIP1 is Downregulated in Hepatocellular Carcinoma by Promoter Hypermethylation

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Author's response to reviews: see over
Dear Editor,

Dear Editor,
Enclosed please find our manuscript that has been revised according to the comments and suggestions of reviewers and editor. The changes in this revised version of the manuscript are stated below point-by-point. We would like to thank in advance for your consideration.

Best regards.

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EDITORIAL REQUESTS:

The manuscript has been copyedited by the professional copy-editor of the Bilkent University. The changes are highlighted as follows:
- Deleted words: colored (pink and red) and strikethrough formatting
- Inserted words: colored (pink and red) and underlined

REVIEWSERS’ REQUESTS:

The concerns of referee 3 are discussed below point-by-point:

However, this reviewer would like to see the whole gel, not just the region surrounding the band labeled SIP1, to see whether the rest of the gel is really devoid of other bands.

A new figure 2 that displays the entire upper part of the gel has been inserted in this revised version. The lower part that was probed with an anti-calnexin antibody shows the equal loading of cell lysates. To our opinion, there are no additional bands as compared to old Figure 2.

Also, there is some concern regarding the position of the band labeled SIP1 relative to the size markers. SIP1 typically migrates at ~140-kDa while this band appears to be in a position of a somewhat larger protein.

140 kDa is the predicted size of SIP1 protein, yet the observed size is somewhat larger (170 kDa) as shown in several papers, including ours in Exp. Mol. Pathol. (2010). Please find below some references:


It would be nice to include a positive control on this gel such as extract from cells transfected with a SIP1 expression plasmid. Might the antibody be cross-reacting with ZEB1, a protein that migrates in SDS-PAGE as if it were ~ 200-kDa.

Below, please find the Figure 1 from our paper in Exp. Mol. Pathol. (Oztas et al. 2010). In part B (right panel), it is clearly shown that 6E5 monoclonal antibody does not recognize endogenous protein upon the knockdown of SIP1 mRNA by a validated siRNA (Qiagene). However, a discrete band appears in the control. In Part C, doxycycline-induced overexpression of SIP1 is detected in the nuclei of A431 cells by immunofluorescence assay. These results clearly showed the specificity of 6E5 antibody for SIP1. Accordingly, this monoclonal antibody recognizes SIP1 specifically and, the SIP1 protein migrates at ~170 kDa in SDS-PAGE.

We would also like to add that this antibody has attracted the attention of the scientific community and antibody companies. 6E5 anti-SIP1 monoclonal antibody is now being licensed by a worldwide renowned company and will be available soon in the market.
Fig.1. Endogenous and induced expression of SIP1 is detected by monoclonal antibodies 1C6 and 6E5. (A) HOS2 cells were transfected with control (Neg-si), SIP1 (SIP1-si) and ZEB1 (ZEB1-si) siRNAs. Proteins were extracted 48 h after transfection and western blot was performed with the indicated antibodies. SIP1 specific band was detected with 1C6 and CUK antibodies. Both SIP1 antibodies did not recognize ZEB1 and can detect endogenous SIP1 efficiently. (B) Control siRNA and SIP1 siRNA transfected HOS2 cell lysates were used to compare the new SIP1 MAbs with 5 different commercial antibodies. The results from polyclonal antibodies (goat or rabbit anti-SIP1) and monoclonal antibodies (mouse anti-SIP1) are presented in left and right panels, respectively. With the exception of 474, all other commercial antibodies were either weak or non-specific. (C) A431/WTSIP1 cells maintained in 2 µg doxycycline for 24 h and stained with 6E5 MAb displayed nuclear SIP1 expression (first row), whereas no staining was observed in uninduced cells with the same antibody (second row).