Author's response to reviews

Title: The calcium-binding protein S100P in normal and malignant human tissues

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Author's response to reviews:

Dear Dr. da-Silva,

Thank you for your letter and the positive response on our manuscript (2850042871247769). We have revised the manuscript based on the reviewer’s remaining comments. We do agree that the manuscript now reads better and hope that you'll find the revised version acceptable for publication in the BMC Clinical Pathology.

After the previous revisions one reviewer still raised concerns about the specificity of the new S100P antibody that is described in the manuscript. He proposed that the specificity could be confirmed by a blocking experiment using various S100 proteins. Unfortunately, it is not possible to accomplish it without an access to different S100 recombinant proteins and antibodies. So this request was not feasible in our situation. To address the reviewer’s concern we performed additional western blot and RT-PCR experiments which were straightforward, suggesting that the new antibody is indeed specific for S100P. The obtained results are shown in the new Figure 2 which replaces the previous photograph. The new western blot clearly shows that the MAb 18-9 reacts with GST-S100P but not with GST alone. It detects S100P only in the cells that display S100P expression in RT PCR and does not give any cross-reaction in MDA-MB 231 cells that do express S100A4 (as also shown in the reviewer’s own paper, ref. 15) and in HeLa cells that express both S100A4 and A2 (ref. 17).

We have made the following modifications to the text:

Pages 7 and 8: The paragraphs ¿Western blotting¿ and ¿Reverse transcription PCR¿ have been modified according to the present experiments.
The western blotting and RT-PCR results have been described as follows: For this purpose, we used a newly generated anti-S100P monoclonal antibody 18-9 produced against a recombinant fusion GST-S100P antigen. First, we verified the specificity of the MAb (Fig. 2). In Western blotting, 18-9 MAb reacted well with the purified GST-S100P protein, but not with the GST alone. The MAb also recognized an 11 kDa protein in HeLa cells and HeLa-R cells transfected with S100P cDNA, whereas no protein was detected in MDA-MB 231 cells and mock-transfected HeLa-R cells (Fig. 2A). These negative cell lines do not express S100P, but show expression of S100A4, as described previously (9,15,17) and also clearly demonstrated in Figure 2B by RT PCR.

Figure 2 and its legend have been replaced with new versions.

Sincerely yours,

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