Author's response to reviews

Title: Psoriasin (S100A7) expression is altered during skin tumorigenesis

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Re: 'Psoriasin (S100A7) expression is altered during skin tumorigenesis. Salem Alowami, Gefei Qing, Ethan Emberley, Linda Snell, and Peter H. Watson

Dear Editor,

We would like to re-submit the attached revised manuscript for consideration for publication in 'BMC Dermatology'. The following pages detail each of the issues and helpful comments raised by the two reviewers and our response to each.

The address for correspondance is to Dr Peter Watson, Dept of Pathology, University of Manitoba, D212-770 Bannatyne Ave, Winnipeg, Manitoba, Canada. R3E 0W3. Tel 204-789-3435, Fax 204-789-3931, email **pwatson@cc.umanitoba.ca**

All authors of this research paper have directly participated in this study and have read and approved the final revised version submitted. The contents of the manuscript have not been published previously and are not now under consideration for publication elsewhere. There are no relevant financial interests.

Yours sincerely,

Dr. Peter Watson, MA., MB BChir., FRCPC
Professor, Dept of Pathology

Detailed response to the issues raised by the two reviewers.

Reviewer JS raises three compulsory revisions. Our responses and corrections are detailed below;

1. "A picture of normal skin …should be included". This is now included as a new figure 1 and the legends to figure 2 have been modified to clarify the identification of structures within panels G&H.
2. The presence of nuclear staining is not an artifact. Psoriasin was originally described to be a secreted protein (Madsen, P et al. Molecular cloning, occurrence, and expression of a novel partially secreted protein "psoriasin" that is highly up-regulated in psoriatic skin. J Invest Dermatol, 97: 701-12, 1991), but we since have found that it also can also be localized in both nuclear and cytoplasmic epithelial cell compartments in normal skin and in breast tumors uisng two different antibodies, irrespective of tissue fixation or immunohistochemistry protocol (Al Haddad, S., et al. Psoriasin (S100A7) expression and invasive breast cancer. Am.J.Pathol., 155: 2057-2066, 1999). The same observation has also been noted by others in skin who noted nucelar as well as cytoplasmic expression with an independent antibody. (Hagens, G., et al Calcium-binding protein S100A7 and epidermal-type fatty acid-binding protein are associated in the cytosol of human keratinocytes. Biochem.J, 339 (Pt 2): 419-427, 1999. and Mol.Cell Biochem., 192: 123-128, 1999). Other secreted S100 proteins have also been localized to cytoplasm and nucleus (Mandinova, A., et al Distinct subcellular localization of calcium binding S100 proteins in human smooth muscle cells and their relocation in response to rises in intracellular calcium. J Cell Sci, 111: 2043-54, 1998). References to the cellular distribution of expression has now been included in the results section.
3. In figure 2A statistical analysis was performed to compare specific individual categories using the non-parametric Mann-Whitney U-test since there is no progression relationship between some of the categories included (ie BCC and squamous lesions).

In addition several discretionary revisions are noted as follows

4. The potential prognostic or therapeutic significance indicated by this study for management of skin tumros is limited. However understanding squamous differentiation in epidermis may be important in tumors arising in other 'epidermal derived' tissues eg breast ducts. A paragraph has been added to the discussion.
5. The question is raised as to whether psoriasin is a secreted protein. We agree with the question and have not addressed this systematically to confirm or refute the original paper. However psoriasin can be readily detected in western blot performed on media from cultured breast cell lines that also show nuclear and cytoplasmic expression of endogenous psoriasin (unpublished data) and the pattern of local stromal staining adjacent to tumor cells that express psoriasin (as observed here and previously in breast tumors (Al Haddad, S., et al. Psoriasin (S100A7) expression and invasive breast cancer. Am.J.Pathol., 155: 2057-2066, 1999) is in keeping with this concept.
6. We were unaware of the Van Ruissen et al FASEB paper when we submitted the paper, but find it very interesting and relevant and have now included it in the references and discussion.

Reviewer KP raises three discretionary revisions. Our responses and corrections are detailed below;

1. “it would have been useful if the authors include a few psoriatic skin lesions for comparision”. We did include several psoriatic skin lesions as positive controls and this has now been noted in the materials & methods and results text and new figure 1.
2. “the authors should clarify that their scoring result was not biased due to differences in tumor cell numbers.” Expression was assessed by semi-quantitative scoring across the entire in-situ or invasive lesion or component. The scoring included the intensity and the proportion of tumor cells staining (not the number of cells) , and was not biased by cell density. This has been clarified in the methods section.
3. We agree that future studies might usefully examine potential associations between psoriasin and additional parameters and genes. As a first step we have now included the results of an assessment of proliferation (based on mitotic count) in these lesions which shows that within normal and pathological squamous epithelium psoriasin is correlated with mitosis (fig 4A).