Author’s response to reviews

Title: SOD2 immunoexpression predicts lymph node metastasis in penile cancer

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Author’s response to reviews: see over

Magdalena Morawska  
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Dear Prof. Morawska,

We are thankful for the critical review of our manuscript and all pertinent suggestions. Please, find below our response to the topics indicated by the reviewers.

Reviewer 1:

“line 82: In the abstract a 44,9% rate of SOD2 positive cases is reported, while a 44,8% SOD 2 positivity rate is quoted in Table I.”

The SOD2 positivity rate is 44,8%. This has been corrected in the abstract.

“line 136: It is not so clear if the authors report “regional recurrence” instead of “lymphnodal metastatic recurrence”. This sentence could be confusing and the authors have to clarify it”

In order to better explain this issue the text between lines 133 and 140 of the Introduction was replaced with: “Since no patient received inguinal or pelvic radiation as part of the treatment, those cases with no lymphadenectomy and no lymphnodal metastatic recurrence in a 3-year follow-up period had their lymph node status classified as “negative” (n=46). Nevertheless, nine men without lymphadenectomy
developed lymphonodal metastatic recurrence during follow-up after penectomy (median time to recurrence: 7.1 months; range: 1.4 – 22.1 months) and consequently they were considered as having “positive nodes” (tumor progression not previously detected).”

“In Conclusion they mention that “implication of SOD 2 expression in thin penile tumors is uncertain..Could the authors claim that SOD2 expression is useful and predictive of lymphonodal metastasis in >mm.5 thick tumors?”

Our data clearly shows that SOD2 expression is an independent predictive factor of inguinal lymph node metastasis in the multivariate model. However, we emphasized that its contribution in clinical practice remains to be determined. This will certainly require SOD2 analysis in a larger number of samples, preferentially, from different medical centers.

Reviewer 2:

“-Page 4, line 103-104: a reference should be provided concerning this statement.”

The reference #6 “Pompeo AC: Extended lymphadenectomy in penile cancer. Can J Urol 2005, (1):30-6; discussion 97-8.” was included to address this particular issue.

“- Page 4, lines 104-107: this phrase is confusing. Please re-phrase”

This part of the text has been re-phrased as follow: “Therefore there is a need to identify other markers that may predict the occurrence of inguinal metastasis, perineural and vascular invasion. The use of these markers could be valuable to better define the subset of patients that will benefit from different therapeutic approaches [2-7].”

“- Page 6, Immunohistochemistry: a polyclonal antibody was used for the purposes of this study. How was the specificity of the antibody tested (western blot? other?). Please clarify.”

Antibody specificity was determined by:
Comparing SOD2 immunoreactivity between different regions of the histological samples analyzed in the study. Since SOD2 is up-regulated in metabolic active cells a different SOD2 staining pattern was expected when comparing normal and tumor tissue. In normal epithelium, either adjacent to SOD2+ or SOD2- tumor tissue, SOD2 immunoreactivity was restricted to the basal and parabasal cell layers. A similar SOD2 staining pattern, using the same antibody, was previously reported by us in cervical tissue samples (Termini L, Filho AL, Maciag PC, Etlinger D, Alves VA, Nonogaki S, Soares FA, Villa LL. Deregulated expression of superoxide dismutase-2 correlates with different stages of cervical neoplasia. Dis Markers. 2011;30(6):275-81. doi:10.3233/DMA-2011-0784.)

Analysis of SOD2 expression was also performed using another rabbit polyclonal antibody from Abcam (Anti-SOD2 antibody #ab13533) with similar results.

"Page 7, line 170: should read "...percentage of stained tumoral cells."
Page 11, line 283: should read "...which is less frequently associated with..."

These modifications have been included as requested.

We hope that in its present revised form this manuscript will be accepted for publication in BMC Clinical Pathology.

Yours sincerely,

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