Reviewer's report

Title: Immunohistochemical expression of Heat Shock Proteins in canine malignant mammary tumours

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Reviewer: Juana Martin de las Mulas

Reviewer's report:

General
Romanucci and colleagues describe the application of immunohistochemistry for heat shock proteins (Hsp) Hsp27, Hsp72, Hsp73 and Hsp90 to 3 normal mammary glands and 30 malignant mammary gland tumours of dogs. They also describe the correlation between the immunohistochemical expression of Hsp and overall survival of dogs. To my opinion, the paper contains some useful information and warrants publication, but not in its present form because: 1) The combined histologic classification and staging method of the tumours is not correct 2) The results are based on the classification and staging of tumours.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

(1) The staging of malignant tumors.
To my opinion, 9 out of 10 stage 0 tumours (see Table 1) have been histologically classified as infiltrating carcinoma according to the WHO classification of Misdorp and colleagues (1999). Thus, although the work of Gilbertson and colleagues (1983) is cited as the source of the tumour staging system (page 7, 1st paragraph), the staging of the tumours does not follow the Gilbertsonâ€™s staging guidelines (see pages 133 and 134 of this work). On the contrary, it is a modification (three vs. four stages, for example) that does not adjust to the original work.

For Gilbertson et al., 1983, â€œstage 0 = malignant epithelial cell proliferation confined to ducts and/or ductules without stromal invasionâ€ . The equivalent sentence of Romanucci et al is: â€œstage 0 = tumours without stromal invasionâ€ . Both statements define a noninfiltrating in situ carcinoma (Misdorp et al 1999), although the later is more imprecise. However, nine out of the 10 histologic types of tumours listed under Stage 0 in Table 1 (numbers 2 to 10) have stromal invasion because they have been histologically classified as carcinomas. So, only number 1 should be placed in this list of stage 0 tumours.

Stage I is more differently defined in the present manuscript when compared to that of the alleged source: â€œtumors with stromal invasionâ€ (present manuscript) vs. â€œinvasive carcinoma limited to local structures without identifiable invasion of blood or lymphatic vesselsâ€ for Gilbertson and others (1983).

Thus, cases 2 to 20 of Table I are stage I tumors according to the Gilbertson staging method. Finally, stage II (cases 21 to 30, Table 1) is the only one defined according to Gilbertson et al (1983) while stage III is lacking.

(2) Some of the histologic types of tumors shown in Table 1 are not present in the Histological classification of mammary tumors of the dog and the cat of Misdorp and others (1999) (as stated in page 7, 1st paragraph).
I find it is very wise to use WHO classifications of tumours of domestic animals to assign the histologic type to neoplasms of different locations because it is very important to use the very same terms in different studies so results can be compared. However, â€œscirrhous carcinomaâ€ is not present in that classification; â€œcystic-papillaryâ€ is a special variant of â€œtubulopapillary carcinomaâ€ ; and histologic patters of growth such as â€œtubularâ€ and â€œsolidâ€ are not completely identified (they are simple carcinomas) (Misdorp et al 1999, page 16).

(3) There is not a single complex carcinoma type included in the study.
This type of tumor is much more frequently found that either noninfiltrating (in situ) carcinoma or simple solid carcinoma. I think it is very important that the authors explain this issue: did they exclude complex carcinoma from the study? If so, why? Further, Figure 6 and probably Figure 7 show nests of proliferating myoepithelial cells. According to the WHO classification, these tumours should have been classified as a complex carcinoma.

(4) The staining of myoepithelial cells with Hsp.
Page 11, lines 4-6: Myoepithelial cells are stained in stage 0 tumours, while they are described as negative in the normal mammary gland (page 11, line 6). As myoepithelial cells of stage 0 (in situ) malignant proliferations are considered to be pre-existing myoepithelial cells (Yaziji et al, Adv Anat Pathol. 7: 100-109,
2000), the change of immunoreactivity pattern should be addressed. 

Page 11, line 6: Describe which components of ducts are negative.

Page 11, lines 9 and 10: What types of lobular structures (alveolar, ductular)? What type of cells (epithelial, myoepithelial)? Where each (cytoplasm, nucleus)?

Page 11, lines 14 and 16: Describe which components of ducts are positive.

Page 12, line 20: Describe they type of tumor where â€œproliferating myoepithelial cellsâ€ were found.

Discussion: It is extremely long and verbose and it does not always refer to own findings. Thus, there are 2 pages and a half dedicated to Hsp 90 expression. Further, the paragraph concerning estrogen receptors (page 20) is pure speculation and should be deleted.

Figures: The histological type of tumour should be identified in all legends for figures.

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

Page 3, lines 3-6: Clarify this sentence because to talk about HSP70 family is contradictory with that of page 2, line 21 where it is said that HSP 73 is reduced.

Page 19, line 11: â€œIntermediate glandular filamentsâ€?

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Discretionary Revisions (which the author can choose to ignore)

Page 7, line 5: Twenty three years ago, availability of cell lineage markers in canine tissue samples using immunohistochemical techniques had not reached the level it has nowadays. Myoepithelial cells (ME) are markers of the malignancy of epithelial cell proliferations because it is considered that an intact ME cell layer identifies benign lesions and in situ carcinoma (depending on the histologic features of the growth).

The lack of the ME cell layer identifies infiltrating carcinoma in humans and dogs (Yaziji et al, Adv Anat Pathol. 7: 100-109, 2000; Espinosa de los Monteros et al Vet Pathol 39; 247-256, 2002; MartÃ­n de las Mulas et al Veterinary and Comparative Oncology 2: 24-35, 2004). To my opinion, the definition of tumour stages (i.e., identification of stromal invasion) in this study should be based in the identification of pre-existing myoepithelial cells using cell lineage markers.

Page 13, line 18: Refer to your findings exclusively. â€œPoor prognosisâ€ is a very wide term that could be based of very different parameters (clinical, histological).

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What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No

Declaration of competing interests:

I declare that I have no competing interests.