Author's response to reviews

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

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Author's response to reviews: see over
Reviewer: Juana Martin de las Mulas

Major Compulsory Revisions

(1) (2) We agree with the reviewer about the inconsistency between our histological classification reported in Table 1 and WHO classification of Misdorp et al. (1999). In fact, it is correct that, according to Misdorp et al. (1999), only in situ carcinoma can be considered as noninfiltrating carcinoma, independently of its histological characteristics and thus, only this type of tumour can be placed in the list of stage 0 tumours according to Gilbertson et al. (1983). Instead, it is the previous WHO classification proposed by Hampe and Misdorp (Bull World Health Organ. 1974, 50:111-133), as well as that subsequently proposed by Misdorp and Hart (J Natl Cancer Inst 1976, 56:779-786), which classified tumours on the basis of their histological types, independently of their infiltrative or noninfiltrative growth. So, we clarify that 9 out of 10 histological types of tumours listed under stage 0 in Table 1 (cases 2 to 10) do not have stromal invasion, as it could be apparently deduced, but they have been only incorrectly indicated on the basis of their particular histological aspect. In fact, they really are noninfiltrating carcinomas according to the definition of Misdorp et al. (1999), which establish that this type of tumours should comprises those lesions that display histological characteristics similar to their invasive counterparts, but that have not invaded through the basement membrane. For all our stage 0 cases, the histological growth pattern permitted to exclude the presence of stromal invasion. Therefore, since we also find that it is wise to adopt the most recent WHO classification of mammary tumours of dog (Misdorp et al. 1999), because it is important to use the same terms in different studies so that results can be compared, we agree that, according to this classification, we can only define all our stage 0 tumours as noninfiltrating (in situ) carcinomas, independently of their prominent histological pattern. Thus, we have modified Table 1 and the corresponding Fig. 9 by correctly defining all stage 0 tumours of the previous version as in situ carcinomas. At the same time, we have removed the term scirrhous carcinoma, which is not present in that classification, and we have replaced it by the term simple carcinoma, since according to Misdorp et al. (1999) this histological type comprises tumours with considerably
variable amount of stroma. Furthermore, as concerns the tumours classified as tubular or solid carcinoma, we have now defined them as simple tubular or simple solid carcinoma, while since cystic-papillary type is a special variant of tubulopapillary carcinoma, it has been indicated as simple tubulopapillary carcinoma, as established by Misdorp et al. (1999). Finally, as far as tumour staging is concerned, we have replaced our previous imprecise definitions of stages with that indicated in the original work of Gilbertson et al. (1983). Besides, as regards the lack of stage III, it does not indicate a modification of the Gilbertson’s staging guidelines. In fact, the absence of stage III (systemic metastasis) is due to our intention of focusing the study on the evaluation of the prognostic and/or therapeutic implications of HSPs expression in malignant mammary tumours which do not yet show clinically evidence of a systemic involvement and for which the identification of prognostic markers or therapeutic targets is more necessary. Indeed, we have also particularly evaluated HSPs expression in neoplastic emboli, since cells with metastatic potential that could give rise to not yet clinically evident micrometastasis are considered the main target of adjuvant cancer therapy.

(3) We have not excluded complex carcinoma from our study, which is present in the list of stage I tumours (Table 1: case 8) and Figure 6 has just been obtained from this case (on the contrary, Figure 7 does not show nests of proliferating myoepithelial cells and it has been obtained from a simple solid carcinoma). However, the number of this type of tumour is so scarce, because the selection of cases has been dictated by the possibility to obtain a complete clinical follow-up and not by the frequency of occurrence of each histological type of tumour in the canine species.

(4) As suggested, we have indicated in Discussion the change of Hsp27 immunoreactivity pattern between myoepithelial cells of normal mammary gland and those ones of in situ (stage 0 of the previous version) tumours: in fact, we agree that this pattern is unexpected, because stage 0 (in situ)
tumours are characterized by an intact myoepithelial cell layer, as in normal tissue. The reason for this particular Hsp27 expression in myoepithelial cells remains to be clarified.

Page 11, line 6: Both intralobular ductules and extralobular ducts were negative (we have added this information in the text) (now Page 10, lines 6-7).

Page 11, lines 9 and 10: We have specified in the text that the immunoreactivity was located in the cytoplasm and nucleus of single epithelial cells or isolated alveolar structures (now Page 10, lines 10-11).

Page 11, lines 14 and 16: We have specified in the text that the extralobular ducts were inconstantly positive (now Page 10, lines 15 and 18).

Page 12, line 20 (now Page 12, line 1): Positive proliferating myoepithelial cells were observed in complex carcinoma (Table 1: stage I, case 8).

Discussion: We agree that it was extremely long, so we have revised it, producing a more concise form. Besides, as suggested, we have deleted the paragraph concerning estrogen receptors.

Figures: As suggested, we have indicated the histological type of tumour in all figure legends.

**Minor Essential Revisions**

Page 3, lines 3-6 (now Page 3, lines 4-6): In order to avoid contradictions, we have modified this sentence.

Page 19, line 11 (now Page 16, lines 22-23): the correct sequence of terms is glandular intermediate filaments, which refer to the intermediate filament proteins expressed preferentially in glandular epithelia.
**Reviewer:** Hiroyuki Taniyama

**Major Compulsory Revisions**

Introduction: As suggested, we have shortened the Introduction and some sentences have been moved to Discussion.

Materials and Methods

Immunohistochemistry

P8, Line 21 (now P8, Lines 1-2): Antibody supplier: DAKO, Glostrup, Denmark (we have added this information in the text).

Results

1. P11, Line 11 (now P10, Line 12): there is a mistake in this sentence. The correct form is “while most of the glandular parenchyma was negative”.

2. P12, Lines 4-5 (now P11, Lines 7-8): We have provided a figure with high-power field, illustrating cytoplasmic positivity in myoepithelial cells.

3. P12, Line 23 (now P12, Line 3): We have provided arrows indicating lymphatic emboli in Fig. 7.

Discussion

1. We have shortened the Discussion, in particular the paragraph concerning Hsp90 expression.

2. P18, Line 3 (now P15, Line 18): We have indicated the reference.

3. P20, Line 9 and Line 13: We have slightly reduced the references of Line 9 (now P17, Line 20), while we have deleted the references of Line 13 together with their relative sentence, as suggested by the other reviewer.
Others

Figures

Fig. 1a, 1b, 1c and 1d: We have provided high-power field figures.