Reviewer’s report

Title: Columnar cell lesions of the canine mammary gland: pathological features and immunophenotypic analysis compared with the human breast

Version: 2 Date: 29 July 2009
Reviewer: Gulisa Turashvili

Reviewer’s report:

- Major Compulsory Revisions

Abstract:
1. The presence of preinvasive (include epithelial hyperplasia and both ductal and lobular in situ carcinomas or just in situ carcinoma if both were found) and invasive lesions (rather than epithelial neoplasia because in situ carcinoma is also epithelial neoplasia) was determined.
2. 61.1% (non-atypical CCLs) and 38.8% (atypical CCLs) do not add up to 100%.

Background:
1. The classification system of CCLs is still unclear and inaccurate. Columnar cell lesions (CCLs), not columnar cell change (CCC), may be lined by several (one, two or more make several) layers of columnar epithelial cells. Based on the number of cellular layers, CCLs are divided into two broad categories: columnar cell change [not columnar cell lesion] (1-2 cell layers) or columnar cell hyperplasia (>2 cell layers). CCC and CCH with cytological atypia are further subclassified as flat epithelial atypia (FEA). Please reword Para 2.
2. Dogs may be a promising model animal for comparative oncology and a clearer account of the alterations in canine mammary cancer may contribute to a better understanding of the key steps in the formation of human tumors but it is unlikely to almost certainly lead to it.

Material and Methods:
1. The authors first mention that the specimens with previous diagnosis of epithelial hyperplasia were selected from the archive but later they describe 60 cases with associated tumors, including 19 cases with invasive tumors. ‘Epithelial hyperplasia’ should be changed to ‘epithelial neoplasia’ or ‘epithelial lesions’. 
2. Please specify how many mammary gland samples were obtained after clinical diagnosis of mammary tumor and surgical removal of the lesion. If this applies to all cases, does it mean that 7 cases with no associated cancer had clinical diagnosis of invasive tumor and underwent surgery but turned out to be CCLs?

3. The classification system of CCLs is unclear: two categories are CCC and CCH (not CCL and CCH), each with or without atypia. Perhaps they authors should add a simple table showing all four subtypes of CCL: CCC without atypia, CCH without atypia, CCC with atypia (FEA), and CCH with atypia (FEA).

4. The CCL classification system was proposed by Schnitt and Vincent-Salomon (Adv. Anat. Pathol. 2003; 10; 113–124). Feeley and Quinn discussed this classification in their review.

5. ‘The association of CCC with malignant and benign lesions….’. CCC should be changed to CCL because this includes all columnar cell lesions.

6. Specify the host for all antibodies (all mouse except for rabbit HER-2).

7. The question about the visualization system enquired a detection system for IHC. Since the authors had mouse and rabbit antibodies, have they used two secondary antibodies - goat anti-mouse and goat anti-rabbit? This should be clarified in the text.

8. Specify which ‘normal serum’ was used in place of primary antibodies for negative controls.

9. Was scoring automated on the Lica-Qwin system? If so, the details about this automated scoring should be provided.

Results:

1. The authors use the term ‘CCC’ in place of ‘CCL’ and vice versa which is rather confusing. A few examples: a) CCCs without atypia were identified in 41 cases. Actually, this is about non-atypical CCLs, ie CCC without atypia (n=39) and CCH without atypia (n=2); b) of 26 FEA (atypical CCLs), 24 are atypical CCCs (not CCL) and 2 are atypical CCHs; c) Sixty cases of the CCLs, not CCCs, showed coexisting neoplastic lesions. Please check the manuscript for other discrepancies (CCLs associated with ductal hyperplasia etc).

2. Of in situ carcinomas, how many were DCIS and LCIS?

3. Specify all associated lesions in six cases subjected to IHC. Also, when discussing IHC results, specify ‘in all six cases studied’ because sample size is rather small for immunophenotypic analysis.

Figures

Fig. 2. This figure shows CCH and it is unclear why more than two cell layers were absent. Why is the absence of architectural pattern emphasized?

Fig. 3. 3A: Acini lined with 1-2 cell layers do not show cytological atypia. Larger acini are the ones with FEA and they seem to be lined by >2 cell layers. As different CCL subtypes may coexist in the same breast, the figure legend should be focused on atypical features. 3B should emphasize the number of cell layers
Fig. 4. 4A is not columnar cell change because there is >2 cell layers. There is high background staining with ER. Please provide more representative figure.

Discussion:
1. In human breast, CCLs have been described under a variety of terms. Have CCLs been completely ignored in canine specimens or they were reported as some kind of epithelial hyperplasia? This should be discussed briefly.

- Minor Essential Revisions

Abstract:
1. The paper describes the immunophenotypic (not molecular) profile of columnar cell lesions.
2. ‘…dilated acini lined with a single layer or more than two cell layers of columnar epithelial cells with elongated nuclei’. Some acini will be lined by two cell layers, so please simply use ‘several cell layer’ when talking about CCLs in general.
3. Change ‘when to comparing’ to ‘when comparing to’.

Methods:
4. ‘analized’ is misspelled.

Results:
5. The second sentence about associated lesions should be moved to where the authors are discussing these associated lesions.
6. Change ‘nuclei that were not regularly oriented perpendicular to the basement membrane’ to ‘nuclei that were not perpendicularly oriented to the basement membrane’.
7. whith is misspelled
8. Is HCC misspelled CCH?
9. Fig. 1. Insert an arrow to indicate the apical snouts.

Discussion:
11. It is unclear what ‘risk biomarkers’ mean.
12. The idea about using dogs as a comparative model is repeated in the last two paragraphs.

- Discretionary Revisions

None

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

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests