Reviewer's report

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

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Reviewer: Gulisa Turashvili

Reviewer's report:

- Major Compulsory Revisions

Abstract:
1. Columnar cell lesions (CCLs) are believed to be a non-obligate, intermediate step in development of some forms of low grade DCIS and invasive carcinoma (AJSP 2005;29:734), ie CCLs may be involved in the development of breast cancer rather than in the progression of already developed breast cancer. The same applies to Para 1 in Background.
2. ‘The proliferation rate as measured by Ki67 appeared higher in the lesions analyzed’. Please clarify the sentence. Are you comparing normal TDLUs with CCLs?

Background:
1. CCLs may be lined (not outlined) by one, two or more (not just one or two) layers of columnar epithelial cells. The number of cellular layers determines whether it is columnar cell change (1-2 cell layers) or columnar cell hyperplasia (>2 cell layers). Also, only non-atypical CCLs show nuclei oriented perpendicular to the basement membrane. The term FEA (flat epithelial atypia) is only used when cytological atypia is present. Please reword Para 2.
2. CCLs may be precursors not only for low-grade DCIS but also invasive carcinoma.
3. It is not clear how ‘a clearer account of the alterations in canine mammary cancer will almost certainly lead to a better understanding of the key steps in the formation of human tumors’.
4. The paper describes the incidence (rather than the presence) of CCLs in canine mammary gland specimens.

Material and Methods:
1. Please specify the lesions associated with CCLs.
2. The abbreviation for Columnar Cell Lesions without atypia would be CCLs not CCC because CCC stands for Columnar Cell Change. FEA includes both columnar cell change (not lesion) with atypia and columnar cell hyperplasia with atypia.
3. None of the antibodies seem to react with canine tissue. Authors should
explain how the immunohistochemistry protocols were optimized. What were the positive and negative control tissues?

4. Please specify exactly which visualization system was used.

5. Since CCLs are not invasive tumor specimens, it is not clear why it is reasonable to use ASCO/CAP guidelines for HER2 scoring. Furthermore, none of the samples were HER2-positive, so HER2 expression could not be scored according to these guidelines.

6. Were these canine tissues selected for mammary lesions (invasive tumors or other lesions?) when the specimens were first collected? In another words, it is important to know whether these CCLs were tumor-associated lesions or randomly sampled mammary tissues. According to Table 1, only 7 samples had no associated tumor.

Results:
1. According to Para , associated lesions were represented only by ductal and lobular hyperplasias but Table 1 includes invasive malignant tumors, in situ carcinomas, and benign tumors. Were CCLs associated with both ductal hyperplasia and invasive, in situ or benign tumors? This should be clarified.

2. What were the associated benign and malignant tumors?

3. What does ‘apically dilated acini’ mean?

4. If five cases of columnar cell change (CCC) showed more than two cell layers, these could not be classified as CCC, because by definition CCC should have one to two layers of columnar epithelial cells. The authors were correct when they classified these 5 cases as CCH without atypia. However, the term columnar cell lesion (CCL) should be used instead of CCC.

5. Were 34#E-12, p53 and HER2 all negative in invasive tumors?

Figures

Fig. 1. According to the figure legend, 1A shows normal mammary acini. Are not all four figures CCCs (1B definitely is a high-power view of the upper right area of 1A)? ‘Normal mammary acini with dilated lumina’ should be changed to ‘dilated mammary acini’ as TDLUs are not normal in CCL.

Fig. 3. Acini lined with 1-2 cell layers do not show cytological atypia. Different CCL subtypes may coexist in the same breast. Therefore, the figure legend should be focused on atypical features.

Fig. 6. There is high background staining with ER and moderate background staining with Ki67. Membranous staining is difficult to appreciate with E-cadherin. Please provide higher resolution figures.

Discussion:
1. Was there any association between CCLs and pregnancy?

2. Myoepithelial/basal and some epithelial cells in human CCLs are positive for CK5/6 (Mod Pathol 2008;21:1413-20). Also, according to the antibody insert sheet, 34#E12 stains all epithelial layers including luminal and basal epithelium
and ductal cells in human breast (Mod Pathol 1997; 10:93A). In our experience, wild-type p53 is also weakly expressed in human CCLs.

3. In human breast, CCLs have been described under a variety of terms including blunt duct adenosis, columnar alteration with prominent apical snouts and secretions, columnar metaplasia, enlarged lobular units with columnar alteration (AJSP 2005;29:105). Have similar lesions been described in dogs? Are there any synonymous terms?

4. What treatment modalities may be tested on canine tissues for CCLs?

5. Please state the limitations of the work such as antibodies not tested on canine tissue etc.

6. More references should be acknowledged.

7. The paper needs some language corrections, for example, ‘They were observed in the terminal duct lobular units and characterized at dilated acini lined with a single layer of columnar epithelial cells with elongated nuclei’ in Abstract; ‘Flat epithelial atypia presence is often associated with in situ carcinomas…’ in Figure 4 legend, etc.

- Minor Essential Revisions

1. Figure 4: Correct the title ‘…with malignant e benign tumors’ and the label on the chart ‘without tumor’

2. UDH stands for usual ductal hyperplasia. Please add it to the abbreviation list.

3. Abbreviation is misspelled.

- 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