Correlation of histological grade and expression of adhesion molecules in canine mammary gland carcinomas

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Abstract

The histological grade is usually used as a prognostic factor in canine mammary gland carcinomas, but the actual biological behaviour is not always in accordance with this available tool. Disrupted expression of cell adhesion molecules is a very promising way how to predict possible tumour spread. The goal of this study was to detect and quantify the expression of adhesion molecule E-cadherin and β-catenin by means of immunofluorescence and relate the findings with the histological grade in 18 samples of canine mammary gland carcinomas.

There is a disruption of β-catenin and E-cadherin expression in canine mammary carcinoma. Significantly positive correlation was found between the expression index of E-cadherin and β-catenin with the histological grade. A significant difference ($P < 0.05$) in the membrane index (MI) of β-catenin expression was found between groups of canine mammary carcinomas (CMCs) grade I and II, grade I and III, grade II and III. A significant difference ($P < 0.05$) in the MI of E-cadherin expression was also found between groups of CMCs grade I and II, grade I and III, grade II and III. A significant difference ($P < 0.05$) in the cytoplasmic index (CI) of β-catenin expression was found between groups of CMCs grade I and II, grade I and III. In the case of CI expression of E-cadherin, no significant difference was found in the expression of E-cadherin in CMCs of different grade. The results of the study show that these adhesion molecules could be promising markers in determining the prognosis of patients with CMCs.

Canine mammary tumours are a heterogenous group of tumours and are the most common oncological disease in bitches (Rezaie et al. 2009; Sleeckx et al. 2011; Sorenmo et al. 2011; Cassali et al. 2014; Furuya et al. 2015; Salas et al. 2015). Canine mammary tumours (CMTs) represent 25–50% of all tumours (Novosad 2003) and more than 82% of tumours of the genital apparatus in bitches (Ettinger and Feldman 2010). Most commonly this disease occurs in older unspayed bitches or in bitches spayed at an older age (Cassali et al. 2014). Determining the histological grade of malignancy is one of the most important prognostic factors. A consensus dealing with this issue was published in 2013 (so called Peña grading system) (Peña et al. 2013). This methodology is now considered as a gold standard in diagnostics, treatment and determining the prognosis.

In comparison with mammary gland carcinomas in women, the treatment of canine mammary carcinomas (CMCs) is based in most cases on surgical removal. A limiting factor for using systemic treatment is the lack of knowledge regarding CMCs. Further studies are necessary to obtain more detailed information on the biology of CMCs.

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Phenotyping based on detection of surface features, so-called markers, allows to determine the type of tumour and get more accurate information regarding the patient's prognosis and set specific treatment according to the patient's individual needs (Cassali et al. 2014).

Adhesion molecules are cell molecules capable of determining the metastatic potential of primary neoplastic lesion by cell adhesion to itself or surrounding tissues. These molecules include cadherins, a group of transmembrane proteins. Cadherins play an important role in intercellular junctions in structurally normal tissues (Kaszak et al. 2018). One of the most frequently investigated adhesion molecules is epithelial cadherin (E-cadherin). The main function of this molecule is adhesion of cell to epithelium (Matos et al. 2006). Loss or reduced expression of adhesion molecules is associated with lower differentiation of neoplastic cells, development of metastases in sentinel lymph nodes, and invasiveness, which has an adverse effect on the patient (Cassali et al. 2014). In epithelial cells, molecule of E-cadherin creates a complex with an intracellular binding domain of β-catenin (Perez-Moreno and Fuchs 2006). Beta-catenin is a molecule with a very complex cellular function; besides the cell adhesion maintenance, it is a key regulator of Wnt signalling pathway (Perez-Moreno and Fuchs 2006; Grigoryan et al. 2008). Wnt signalling plays a very important role in tumour development and even though it is extensively studied in the human medicine, the research of this pathway is still at the beginning in veterinary oncology (Chon et al. 2013; Yu et al. 2017; Putnová et al. 2021) In a simplified manner, the dislocation of β-catenin from the membrane to the cell cytoplasm or nucleus can be a sign of activation of this pathway (Yokoya et al. 1999; Luo et al. 2018). Not only the loss of cadherins, but also the disruption of the cadherin/catenin complex leads to epithelial integrity disruption and possible tumour spread. Therefore, we decided to determine these expressions of both of these molecules together.

Immunohistochemical methods (IHC) or immunofluorescence methods (IF) were used to determine these molecules. The principle of IF is the binding of diagnostic antibodies with a conjugate with fluorescent dye (fluorochrome) to antigens in tissues. This binding can be demonstrated with a fluorescence microscope (for example under ultraviolet radiation) (Cammack et al. 2011). The main difference between these two methods is that in case of IF the detection is done using immunofluorescence, while IHC is based on chemical reaction to detect monoclonal and polyclonal antibodies. The advantage of IF is higher specificity and sensitivity in comparison to IHC.

The goal of this study was to detect and determine the membrane and cytoplasmic index of expression of adhesion molecules E-cadherin and β-catenin in CMC using immunofluorescence depending on the grade of malignancy.

Materials and Methods

Samples of mammary gland tumours obtained surgically at the Department of Surgery and Orthopaedics at the Small Animal Clinic, VETUNI Brno, from the year 2016 to 2021 in the form of native tissue of mammary gland were used for histopathological analysis. Only samples from patients undergoing surgery only were included in analysis; patients in which adjuvant chemotherapy was used were excluded. Only samples from patients with known survival time were used for the final statistical analysis.

Samples were labelled and fixed in 10% buffered formaldehyde solution for 48 h at the Department of Pathological Morphology and Parasitology, VETUNI Brno. Tissue marking dyes were used to label margins (Histological Davidson Marking System®, Bradley Products, Bloomington, USA). Representative parts of the lesion were selected and processed into the form of paraffin blocks using tissue processor (Leica TP 1020, Leica Biosystems, Illinois, USA). Standard histological sections were made of 3–5 μm in thickness. These sections were then dyed with haematoxylin and eosin stain. At the same time, serial sections were prepared on SuperFrost® slides (Thermo Fisher Scientific, Waltham, USA) for immunohistochemical and immunofluorescence analyses. The specimens were then independently evaluated by two veterinary pathologists. Histopathological diagnosis was determined using Goldschmidt classification (Goldschmidt et al. 2011). In every specimen a grade was determined using Peña grading system (Peña et al. 2013).

For immunofluorescence, the double labelling method was used. The primary antibodies used were β-catenin in concentration 1:100 (Cell Signaling Technology, Massachusetts, USA, No 9582), E-cadherin in concentration 1:100 (Thermo Fisher Scientific, No CF800671), as a secondary antibody Alexa Fluor 488 in concentration 1:200 (Thermo Fisher Scientific, No A11008) and Alexa Fluor 565 in concentration 1:200 (Thermo Fisher Scientific, No A11004) were used. The nuclei were counterstained with Draq5™ (Thermo Fisher Scientific, No 65-0880-92). The antigen retrieval was performed in citric acid, pH6 (20 min, hot bath 90 °C). The slides were observed under the confocal microscope Leica TCS SP8 (Leica Microsystems, Wetzlar, Germany). The pictures were edited in Adobe Photoshop 2021 (Adobe, San Jose, USA).

Membrane and cytoplasmic expression of E-cadherin was subjectively evaluated according to the degree of intensity f (f0: negative (0), f1 weakly positive (+), f2: moderately positive (++), f3: strong positive (+++)). The percentage representation of individual groups according to the degree of expression was determined. The result quantification was obtained according to the Lipponen and Collan's (1992) formula and membrane expression index (MI 0–3) and the cytoplasmic expression index (CI 0–3) was determined.

$$I = (f0 * f1) + (1 * f2) + (2 * f3) + (3 * f4) / 100$$

The same procedure was used to evaluate the expression of β-catenin. The quantification according to the Lipponen and Collan (1992) was also carried out. The membrane expression index (MI 0–3) and the cytoplasmic expression index (CI 0–3) was determined.

The software STATISTICA CZ Version 6 (StatSoft CR, Prague, Czech Republic) was used for statistical analysis. Comparisons were made at a significance level $P < 0.05$.

Results

Histological classification

Histologically, all samples were classified as carcinomas (n = 18). The histological subtypes of CMCs were classified as solid carcinoma (n = 4), tubular carcinoma (n = 2), intraductal papillary carcinoma (n = 1), tubulopapillary carcinoma (n = 11). The histological grade of malignant CMCs included grade I (n = 6), grade II (n = 6), and grade III (n = 6).

Disruption of β-catenin and E-cadherin expression in canine mammary carcinoma

In some cases, the expression of E-cadherin was lost in mammary gland tumours. The most obvious changes in the expression were seen in the areas of tumour "budding" and its invasive front. Changes in β-catenin expression were also associated with the loss of E-cadherin signal.

We observed a partial loss of β-catenin on the membranes of canine mammary gland carcinoma. The most striking changes were seen in the expression pattern, which changed from a linear membranous pattern to a clustered and uneven pattern.

In a mammary gland carcinoma grade I we saw that the expression of β-catenin and E-cadherin was preserved. The signal of both markers was membranous with no significant clustering (Plate I, Fig. 1).

In mammary gland carcinomas grade II and III a major change in the expression of E-cadherin and β-catenin was apparent. The expression pattern changed from linear membranous to uneven clustered to even interrupted (Plate I, Fig. 2 and Plate II, Fig. 3).

Significant differences in the β-catenin and E-cadherin membrane and cytoplasmic expression index were found between the histological grades of canine mammary carcinoma. By comparing the membrane index of β-catenin expression in grade I and II mammary gland carcinomas, a significantly higher expression ($P = 0.0028$) was found in the group of grade I tumours. Similarly, a significantly higher expression of β-catenin was found in grade I mammary gland carcinomas compared to grade III ($P = 0.0001$). Significantly higher expression of β-catenin was also found in patients with grade II mammary gland tumours compared to grade III (Fig. 4).

When comparing cytoplasmic expression index of β-catenin in grade I and II mammary gland carcinomas, a significantly higher ($P = 0.049$) expression was determined in the group of grade I tumours. Significantly higher ($P = 0.021$) expression of cytoplasmic β-catenin was found also when comparing grade I and grade III mammary gland carcinomas, where the expression was higher in grade I. When comparing the difference in expression

of cytoplasmic β-catenin in grade II and III mammary gland carcinomas, no significant difference was determined ($P = 0.2818$) (Fig. 5).

The membrane expression index of E-cadherin in the group of grade I mammary gland carcinomas was not significantly different from that of grade II mammary gland carcinomas ($P = 0.387$). By comparing the membrane expression index of E-cadherin in grade II and grade III mammary gland carcinomas, a significantly higher ($P = 0.0001$) expression was determined in patients with mammary gland carcinomas grade II. Similarly, by comparing the membrane expression index of E-cadherin in grade I and grade III mammary gland carcinomas, a significantly higher ($P < 0.0001$) E-cadherin expression was found in patients with grade I mammary gland tumours (Fig. 6).

In case of cytoplasmic expression index of E-cadherin, no significant difference was found when comparing the different grades of mammary gland carcinomas: grade I vs. grade II ($P = 0.3605$), grade I vs. grade III ($P = 0.3144$), and grade II vs. grade III ($P = 0.1657$) (Fig. 7).

In our study a significant positive correlation was determined between the detected adhesion molecules (E-cadherin and β-catenin) and the comparison to their expression index with the histological grade according to grading system Peña et al. (2013). In well differentiated mammary gland carcinomas these molecules were detected to a greater extent than in carcinomas with lower degree of differentiation.
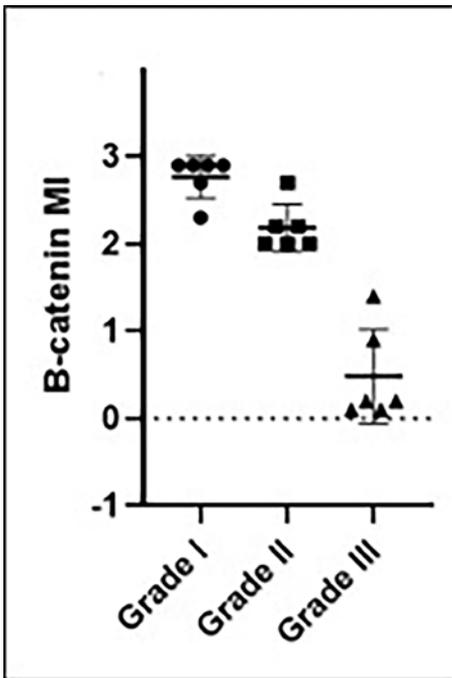

Fig. 4. The relationship between the membrane index (MI) of β-catenin expression (0–3) in patients with mammary gland tumour and the histological grade of malignancy according to Peña et al. (2013)

Fig. 5. The relationship between the cytoplasmic index (CI) of β-catenin expression (0–3) in patients with mammary gland tumour and the histological grade of malignancy according to Peña et al. (2013)

Fig. 6. The relationship between the membrane index (MI) of E-cadherin expression (0–3) in patients with mammary gland tumour and the histological grade of malignancy according to Peña et al. (2013)

Fig. 7. The relationship between the cytoplasmic index (CI) of E-cadherin expression (0–3) in patients with mammary gland tumour and the histological grade of malignancy according to Peña et al. (2013)
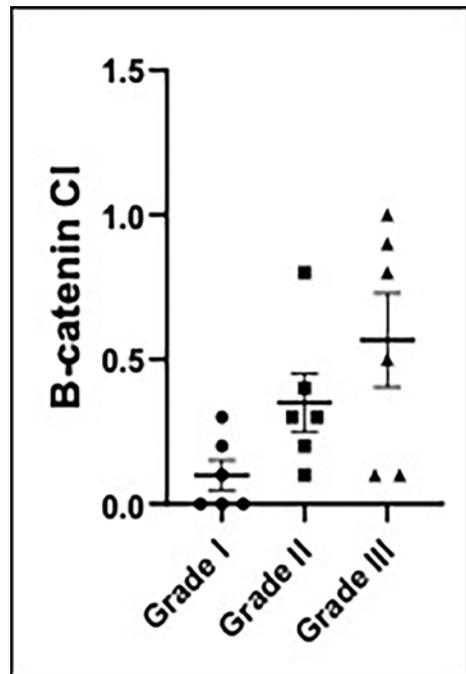
Discussion

Adhesion molecules E-cadherin and β-catenin are expressed mainly by epithelial cells. Expression of other types of cadherins (e.g. P-cadherin) is limited to myoepithelial cells (Restucci et al. 1997). Therefore, our study includes only specimens of mammary gland carcinomas without mesenchymal components. In our study a strong membranous immunostaining of E-cadherin in mammary gland carcinomas grade I was evident. These findings are in accordance with a similar study of Reis et al. (2003), where immunohistochemical methods were used. This outcome is supported by a number of studies in human medicine. In case of well differentiated carcinomas of various organs including mammary gland tumours, there is a marked expression of E-cadherin. Its prognostic potential lies in suppressing invasiveness when it is strongly expressed (Frixen et al. 1991; Oka et al. 1993; Takeichi 1993; Wong and Gumbiner 2003; Pohlodek et al. 2016).

Some studies claim that a lower degree of E-cadherin expression is associated with the presence of less differentiated breast carcinomas in females, but it is not possible to reliably determine to what extent this fact is related to a shorter survival time of patients (Soler et al. 1999; Knudsen and Wheelock 2005; Gould Rothberg and Bracken 2006). The use of E-cadherin as a prognostic marker is still controversial and further studies are needed.

In our study we focused not only on the E-cadherin itself, but also on the E-cadherin/β-catenin complex. This was studied in breast cancer in women and a reduction or loss of the E-cadherin/β-catenin complex was reported a long time ago (Gamallo et al. 1993; Yoshida et al. 2001)

In this study the molecules E-cadherin and β-catenin were detected using indirect immunofluorescence. The main benefit of this method is higher sensitivity and signal amplification in comparison to immunohistochemical methods, which are standardly used to detect these adhesion molecules and various other molecules as well (Im et al. 2019). Another advantage of using this method is multiplexing. In future studies this feature could enable joint localization studies, requiring multiantigen imaging with high definition. Higher image quality is achieved by using confocal microscopes, as was the case in our study. This allows us to avoid blurred images for which the chromogenic enzymes precipitates are responsible. While the enzymatic approach of chromogenic methods limits the quantitative possibilities of immunochemical analyses, fluorescent probes enable high performance and quantitative automated approaches (Cammac et al. 2011; Im et al. 2019). In our study the level of fluorescence intensity was evaluated using the index of membrane or cytoplasmic expression, both in the case of E-cadherin and β-catenin.

Similar work was conducted on canine tissue samples by Brunetti et al. (2005) using immunohistochemistry. Despite the different methodology, the results were similar to those found in our study. The authors also demonstrated a significant correlation of these two markers in the same types of tumours as in our study. Brunetti et al. (2005) found a connection between the expression of both individual E-cadherin, individual β-catenin and joint determination of the expression of E-cadherin and β-catenin. Decreased expression of E-cadherin, β-catenin, or a combination of both was significantly associated with progression from non-infiltrating to highly infiltrating mammary gland tumours. However, according to this study, the expression of the markers had no effect on the length of survival or proliferative activity (Brunetti et al. 2005).

In our study a significant association was found between the expression of E-cadherin and β-catenin in mammary gland carcinomas, depending on their degree of malignancy according to the histological grade. A limitation of this work is the small number of patients included, which is planned to be improved in our further studies.

In conclusion, determination of E-cadherin/β-catenin complex expression in canine mammary carcinomas is a promising supplementary diagnostic method, but further investigation is needed, especially to uncover the role of β-catenin cellular localisation and its link to the tumour biological behaviour.

References

Brunetti B, Sarli G, PreziosI R, Monari I, Benazzi C 2005: E-cadherin and beta-catenin reduction influence invasion but not proliferation and survival in canine malignant mammary tumors. Vet Pathol **42**: 781-787

Cammack R, Atwood T, Campbell P, Parish H, Smith A, Vella F, Stirling J 2011: Oxford Dictionary of Biochemistry and Molecular Biology. 2nd edn, Oxford University Press, 736 p.

Cassali GD, Lavalle GE, Ferreira E, Lime AE, De Nardi AB, Ghever C, Sobral RA, Amorim RL, Oliveira LO, Sueiro FAR, Beserra HEO, Bertagnolli AC, Gamba CO, Damasceno KA, Campos CB, Araujo MR, Campos LC, Monteiro LN, Nunes FC, Horta RS, Reis DC, Luvizotto MCR, Magalhães GM, Raposo JB, Ferreira AMR, Tanaka NM, Grandi F, Ubukata R, Batschinski K, Terra EM, Salvador RCL, Jark PC, Delecrodi JER, Nascimento NA, Silva DN, Silva LP, Ferreira KCRS, Frehse MS, Di Santis GW, Silva EO, Guim TN, Kerr B, Cintra PP, Silva FBF, Leite JS, Mello MF V, Ferreira M De LG, Fukumasu H, Salgado BS, Torres R 2014: Consensus for the diagnosis, prognosis and treatment of canine mammary tumors - 2013. Braz J Vet Pathol **7**: 38-69

Chon E, Thompson V, Schmid S, Stein TJ 2013: Activation of the canonical Wnt/β-catenin signalling pathway is rare in canine malignant melanoma tissue and cell lines. J Comp Pathol **148**: 178-187

Ettinger SJ, Feldman EC 2010: Textbook of Veterinary Internal Medicine: Diseases of the Dog and the Cat. 7th edn, St. Louis. Missouri: Saunders Elsevier, 2218 p.

Frixen UH, Behrens J, Sachs M, Eberle G, Voss B, Warda A, Löchner D, Birchmeier W 1991: E-cadherin-mediated cell-cell adhesion prevents invasiveness of human carcinoma cells. J Cell Biol **113**: 173-185

Furuya M, Funasaki M, Tani H, Sasai K 2015: Identification of novel tumour-associated antigens in canine mammary gland tumour. Vet Comp Oncol **13**: 194-202

Gamallo C, Palacios J, Suarez A, Pizarro A, Navarro P, Quintanilla M, Cano A 1993: Correlation of E-cadherin expression with differentiation grade and histological type in breast carcinoma. Am J Pathol **142**: 987-993

Goldschmidt M, Peña L, Rasotto R, Zappulli V 2011: Classification and grading of canine mammary tumors. Vet Pathol **48**: 117-131

Gould Rothberg BE, Bracken MB 2006: E-cadherin immunohistochemical expression as a prognostic factor in infiltrating ductal carcinoma of the breast: a systematic review and meta-analysis. Breast Cancer Res Treat **100**: 139-148

Grigoryan T, Wend P, Klaus A, Birchmeier W 2008: Deciphering the function of canonical Wnt signals in development and disease: conditional loss- and gain-of-function mutations of beta-catenin in mice. Genes Dev **22**: 2308-2341

Im K, Mareninov S, Diaz MFP, Yong WH 2019: An introduction to performing immunofluorescence staining. Methods Mol Biol **1897**: 299-311

Kaszak I, Ruszczak A, Kanafa S, Kacprzak K, Król M, Jurka P 2018: Current biomarkers of canine mammary tumors. Acta Vet Scand **60**: 66

Knudsen KA, Wheelock MJ 2005: Cadherins and the mammary gland. J Cell Biochem **95**: 488-496

Lipponen PK, Collan Y 1992: Simple quantitation of immunohistochemical staining positivity in microscopy for histopathology routine. Acta stereol **11**: 125-132

Luo Y, Li M, Zuo X, Basourakos SP, Zhang J, Zhao J, Han Y, Lin Y, Wang Y, Jiang Y, Lan L 2018: β-catenin nuclear translocation induced by HIF-1α overexpression leads to the radioresistance of prostate cancer. Int J Oncol **52**: 1827-1840

Matos AJ, Lopes C, Carvalheira J, Santos M, Rutteman GR, Gärtner F 2006: E-cadherin expression in canine malignant mammary tumours: relationship to other clinico-pathological variables. J Comp Patho **134**: 182-189

Novosad AC 2003: Principles of treatment for mammary gland tumors. Clin Tech Small Anim Prac **18**: 107-109

Oka H, Shiozaki H, Kobayashi K, Inoue M, Tahara H, Kobayashi T, Takatsuka Y, Matsuyoshi N, Hirano S, Takeichi M 1993: Expression of E-cadherin cell adhesion molecules in human breast cancer tissues and its relationship to metastasis. Cancer Res **53**: 1696-1701

Peña L, De Andrés, PJ, Clemente M, Cuesta P, Pérez-Alenza MD 2013: Prognostic value of histological grading in noninflammatory canine mammary carcinomas in a prospective study with two-year follow-up. Vet Pathol **50**: 94-105

Perez-Moreno M, Fuchs E 2006: Catenins: keeping cells from getting their signals crossed. Dev Cell **11**: 601-612

Pohlodek K, Tan YY, Singer CF, Gschwantler-Kaulich D 2016: Cadherin-11 expression is upregulated in invasive human breast cancer. Oncol Lett **12**: 4393-4398

Putnová B, Putnová I, Škorič M, Buchtová M 2021: The expression of selected Wnt pathway members (FZD6, AXIN2 and β-Catenin) in canine oral squamous cell carcinoma and acanthomatous ameloblastoma. Animals **11**: 1615

Reis AL, Carvalheira J, Schmitt FC, Gärtner F 2003: Immunohistochemical study of the expression of E-cadherin in canine mammary tumours. Vet Rec **152**: 621-624

Restucci B, Papparella S, De Vico G, Maiolino P 1997: E cadherin expression in normal and neoplastic canine mammary gland. J Comp Pathol **116**: 191-202

Rezaie A, Tavasoli A, Bahonar A, Mehrazma M 2009: Grading in canine mammary gland carcinoma. Biol Sci **9**: 333-338

Salas Y, Márquez A, Diaz D, Romero L, Seagroves T 2015: Epidemiological study of mammary tumors in female dogs diagnosed during the period 2002-2012: A growing animal health problem. PLOS ONE **10**.

Sleeckx N, De Rooster H, Veldhuis Kroeze, EJB, Van Ginneken C, Van Brantegem L 2011: Canine mammary tumours, an overview. Repro Domest Anim **46**: 1112-1131

Soler A, Knudsen KA, Salazar H, Han AC, Keshgegian AA 1999: P-cadherin expression in breast carcinoma indicates poor survival. Cancer **86**: 1263-1272

Sorenmo KU, Rasotto RV, Zappulli V, Goldschmidt MH 2011: Development, anatomy, histology, lymphatic drainage, clinical features, and cell differentiation markers of canine mammary gland neoplasms. Vet Pathol **48**: 85-97

Takeichi M 1993: Cadherins in cancer: implications for invasion and metastasis. Curr Opin Cell Biol **5**: 806-811

Wong AST, Gumbiner BM 2003: Adhesion-independent mechanism for suppression of tumor cell invasion by E-cadherin. J Cell Biol **161**: 1191-1203

Yokoya F, Imamoto N, Tachibana T, Yoneda Y 1999: beta-catenin can be transported into the nucleus in a Ran-unassisted manner. Mol Biol Cell **10**:1119-1131

Yoshida R, Kimura N, Harada Y, Ohuchi N 2001: The loss of E-cadherin, alpha- and beta-catenin expression is associated with metastasis and poor prognosis in invasive breast cancer. Int J Oncol **18**: 513-520

Yu F, Rasotto R, Zhang H, Pei S, Zhou B, Yang X, Jin Y, Zhang D, Lin D 2017: Evaluation of expression of the Wnt signaling components in canine mammary tumors via RT2 Profiler PCR Array and immunochemistry assays. J Vet Sci **18**: 359-367
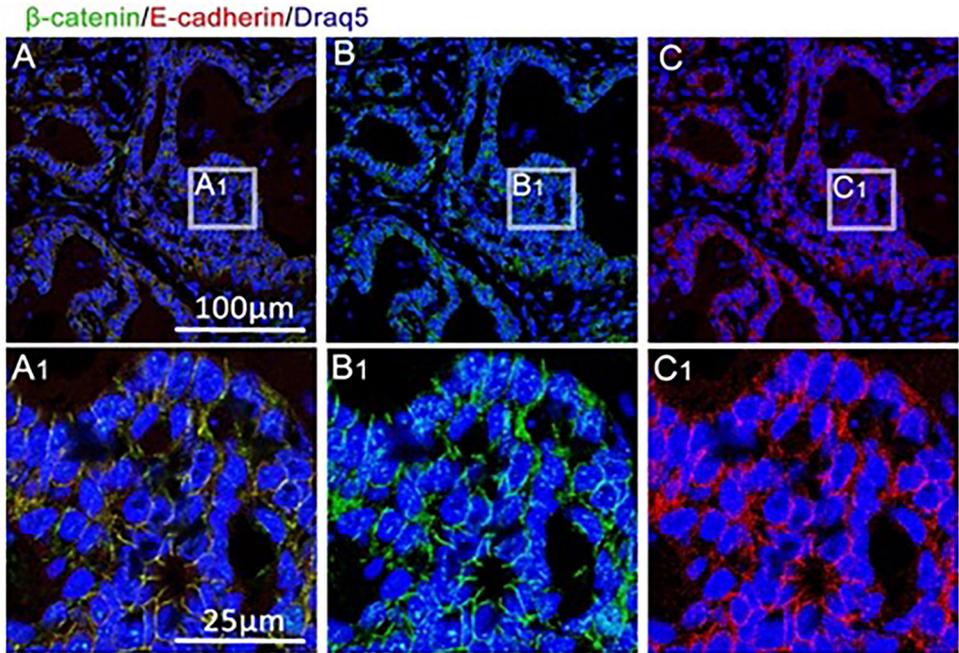
Plate I
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β-catenin/E-cadherin/Draq5

Fig. 1. The expression pattern of β-catenin and E-cadherin in canine mammary grade I (Peña et al. 2013). Sample A, B, C, detailed view A1, B1, C1
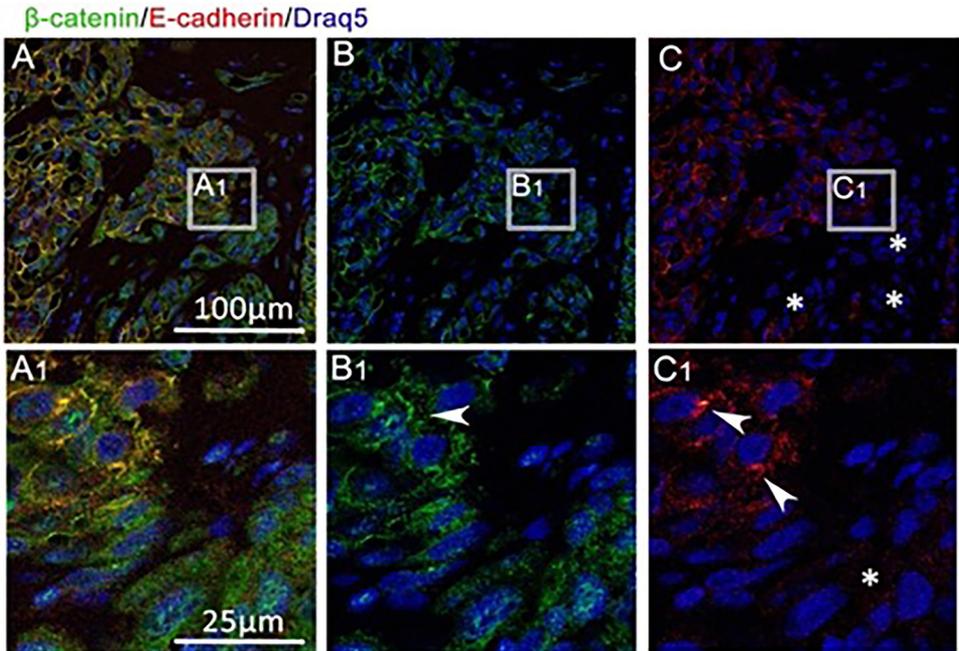
β-catenin/E-cadherin/Draq5

Fig. 2. The expression pattern of β-catenin and E-cadherin in canine mammary carcinoma grade II (Peña et al. 2013). Sample A, B, C, detailed view A1, B1, C1
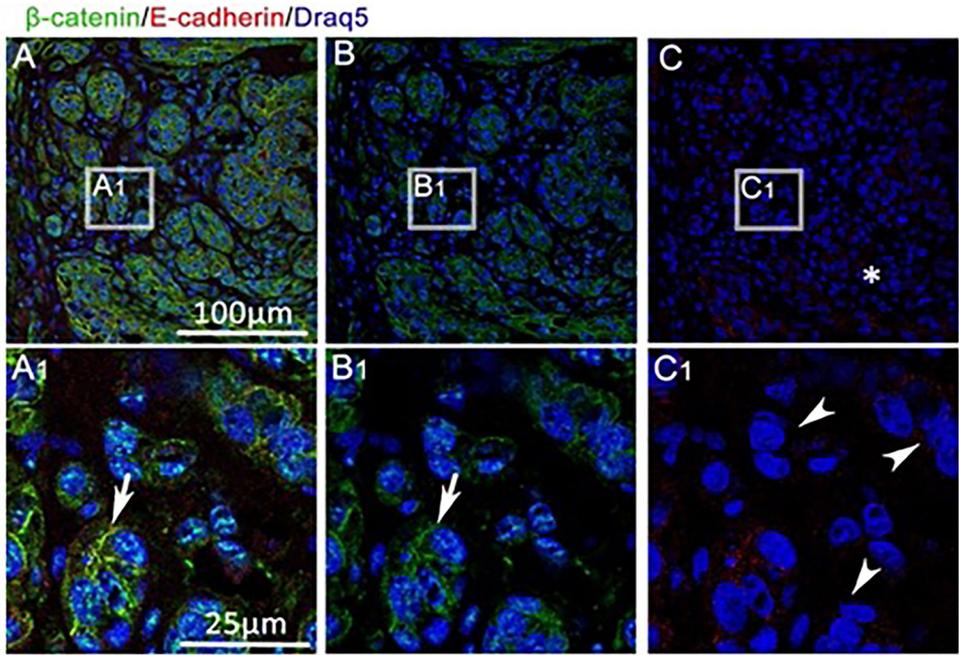
Plate II

Fig. 3. The expression pattern of β-catenin and E-cadherin in the invasive front of the canine mammary carcinoma grade III (Peña et al. 2013). Sample A, B, C, detailed view A1, B1, C1