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PATHOLOGY

Expression of EGF receptors in canine prostate with proliferative inflammatory atrophy and carcinoma

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ABSTRACT: *Gene expression of ErbB1 and ErbB2, and immunostaining of EGFR (Her1) and Her2 (c-erbB-2) were evaluated in this study to ascertain whether these receptors are involved in the evolution of canine premalignant and malignant prostatic lesions, as proliferative inflammatory atrophy (PIA) and prostatic carcinoma (PC). With regards to the intensity of EGFR immunostaining, there was no difference between normal prostatic tissue and tissues with PIA or PC. In relation to Her2 immunostaining, there were differences between normal prostatic tissue and those with PIA and PC, as also differences between prostates with PIA and PC. There was no correlation between EGFR and Her2 immunostaining. ErbB1 gene product was detected in two normal tissue samples, in one with PIA, and in all samples with PC. ErbB2 mRNA was recorded in two canine samples with PIA, in all with PC, but was not detected in normal prostatic tissue. It was concluded that EGFR and Her2 play roles in canine PIA and PC, suggesting that those receptors may be involved in canine prostatic carcinogenesis.*
Key words: *growth factor receptor, Her1, c-erbB-2, PIA, RT-PCR.*

Expressão de receptores EGF na próstata canina com atrofia inflamatória proliferativa e carcinoma

RESUMO: *A expressão gênica de ErbB1 e ErbB2 e a imunomarcação de EGFR (Her1) e Her2 (c-erbB-2) foram avaliadas para verificar o envolvimento desses receptores em lesões pré-malignas e malignas da próstata canina, como a atrofia proliferativa inflamatória (PIA) e o carcinoma prostático (PC). Em relação à intensidade de imunomarcação para EGFR, não houve diferença entre o tecido prostático normal e com PIA e PC. Em relação a Her2, observou-se diferença de imunomarcação entre o tecido prostático normal e aqueles com PIA e PC e entre os com PIA e PC. Não houve correlação entre EGFR e Her2. O gene ErbB1 foi detectado em duas amostras normais, uma de PIA e em todas as amostras de PC. O gene ErbB2 foi detectado em duas amostras de PIA e em todas as amostras de PC, não sendo detectado no tecido prostático normal. Conclui-se que EGFR e Her2 atuam nas lesões de PIA e PC, sugerindo o envolvimento destes na carcinogênese da próstata canina.*
Palavras-chave: *receptor de fator de crescimento, Her1, c-erbB2, PIA, RT-PCR.*

INTRODUCTION

The canine prostate has been extensively studied because of its similarities with the human prostate gland regarding natural occurrence of diseases and hormonal influence in their development, as in prostatic carcinoma (PC) (LEROY & NORTHRUP, 2009). The proliferative inflammatory atrophy (PIA) is a dysplastic change that was first described in the study of the human prostate, and was considered a potentially premalignant lesion (PUTZI & DE MARZO, 2000; WANG et al., 2009). In dog prostate, TOLEDO et al. (2010) have described the histological aspects of PIA.

The ErbB family is a group of tyrosine kinase receptors comprised by Her1 (epidermal growth factor receptor - EGFR), Her2 (c-erbB-2), Her3 (c-erbB-3), and Her4 (c-erbB-4). They are coded by ErbB1 (erythroblastic leukemia viral oncogene homolog), ErbB2, ErbB3, and ErbB4, respectively, and are widely expressed in epithelial tissues where they play crucial roles in regulation of cell differentiation, proliferation, and survival (CIARDELLO & TORTORA, 2008).

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EGFR has an extracellular ligand-binding domain, a transmembrane domain, and an intracellular domain with tyrosine kinase activity for signal transduction. Domains are activated through binding with the epidermal growth factor (EGF) or transforming growth factor alpha (TGF-α), which results in dimerization with Her1, or heterodimerization with another ErbB member. This leads to a receptor-linked tyrosine kinase activation and results in a signaling cascade that produces effects such as cell migration, maturation, differentiation, metastasis, angiogenesis, and inhibition of apoptosis (CARLSSON et al., 2013). EGFR is expressed or overexpressed in solid tumors, including PC. Furthermore, it has also been associated with advanced tumor stages, poor prognosis, and resistance to therapies (CIARDELLO & TORTORA, 2008).

Homodimers are less mitogenic than heterodimers. Since Her2 receptors are preferably heterodimers, they have more tyrosine kinase activity and tend to be more potent, generating more intense intracellular signals, which gives increased mitogenic potential. Therefore, levels of other ErbB family receptors, especially HER2, can significantly affect the EGFR signaling in cancer (YARDEN & SLIWKOWSKI, 2001). Her2 overexpression has been reported in human PC (CARLES et al., 2004; BARTLETT et al., 2005). By contrast, it has also been reported as there being almost no expression of Her2 (LIU et al., 2001).

In domestic animals, EGFR immunostaining has been reported in canine breast tumors (GAMA et al., 2009), primary brain tumors (LIU et al., 2001), nasal carcinomas (SHIOMITSU et al., 2009), lung carcinomas (SABATTINI et al., 2014), and gastric tumors (TERRAGNI et al., 2014). Likewise, cases of Her2 overexpression were reported in 19-35% of canine mammary tumors (HSU et al., 2009; RESSEL et al., 2013), and in 57.9% of canine gastric neoplasms (TERRAGNI et al., 2014). However, there are no reports regarding the expression of both EGFR and Her2 receptors in the canine prostate. Thus, in order to understand molecular mechanisms involving those receptors in canine premalignant and malignant prostatic tissue, the RNA detection of ErbB1 and ErbB2, and the immunostaining of EGFR and Her2 were evaluated in the prostate of dogs with PIA and PC.

MATERIALS AND METHODS

Histomorphological evaluation of 70 canine prostate samples in HE staining was carried out to identify normal prostatic tissue, and those with PIA (TOLEDO et al., 2010), and PC (LAI et al., 2008). Prostatic samples were obtained from no neutered, mixed-breed, adult dogs, and all normal prostatic tissues were harvested from dogs with nonmalignant lesions in the gland at microscopy screening.

The samples selected on histomorphological evaluation were submitted to tissue microarray (TMA) technique according to RUBIN et al. (2002). Immunohistochemistry was performed in the TMA slides. Endogenous peroxidase and nonspecific proteins were blocked with 3% hydrogen peroxide, protein block (Leica, Newcastle, #RE7102) for EGFR, and milk powder 10% (Molico®, Brazil - 10g / 100mL distilled in water) for Her2 (c-erbB-2). Antigen retrieval was performed with citrate buffer pH 6.0, in a pressure cooker for EGFR and in a water bath for Her2 (c-erbB-2). Slides were incubated overnight at 4°C with monoclonal mouse anti-EGFR antibody (Leica, Newcastle, #NCL-L-EGFR-384), clone EGFR-25, diluted at 1:50, and polyclonal rabbit anti-c-erbB-2 oncoprotein (Dako, #A0485), diluted at 1:200. A polymer kit (New Link Max Polymer, #RE7260-K), DAB chromogen and HRP substrate for signal detection were used. The sections were counterstained with Mayer's hematoxylin, washed, dehydrated, cover slipped, and examined by light microscopy.

A sample from normal human placenta (EGFR) and human mammary carcinoma (c-erbB-2) were used as positive control. For negative control, PBS buffer was used in the replacement of primary antibodies. The EGFR and Her 2 (c-erbB-2) immunolabeling were scored according to criteria adapted from BILOUS et al. (2003). Samples scored as 0 and 1+ were assigned as normal expression, and those scored as 2+ and 3+ were considered overexpressed for EGFR and Her2.

The RecoverAll™ Total Nucleic Acid Isolation Kit (Life technologies, #AM1975) was used to extract total nucleic acids (RNA and DNA) from all FFPE tissue samples used in the TMA. Twenty sections with 12μm from each paraffin block were deparaffinized using xylene and ethanol washes, followed by protein digestion with protease K, and incubation in water bath during 16 hours at 50°C, and 15 minutes at 80°C. Sequentially, RNA isolation and purification samples were treated with DNase mix followed by washing and finishing with the solution. The yield and quality were measured by NanoVue™ Plus Spectrophotometer (GE Healthcare, - 28-9301-69 AC). The yield was estimated by UV absorbance at 260nm, and the quality in rates between A260/A280, using samples higher than 1.70. Likewise, the presence of RNA was confirmed by 1%

agarose gel electrophoresis on PowerPac™ Basic Power Supply (Bio-Rad, #164-5050), and the images of gels were obtained by Gel Doc™ EZ System (Bio-Rad, #170-8270).

The cDNA synthesis was performed using a High Capacity RNA-to-cDNA Kit (Applied Biosystems®:#4387406). The polymerase chain reaction (PCR) was performed in duplicate for cDNA amplification of ErbB1 - (GenBank: AY527212.1) - *Canis lupus familiaris* EGFR, locus 18; ErbB2 (GenBank: AB008451.1) - *C. l. f.* ErbB2 - locus 9; and beta-actin as positive control (housekeeping gene - GenBank: AF021873.2) - *C. l. f.* actin, gamma 1 (ACTG1) locus 9. PCR amplified gene fragments with 149pb, 157pb, and 151pb.

In the PCR microtube were inserted cDNA; PCR buffer; $MgCl_2$; dNTP (Life Technologies™ #10297-018); oligonucleotide primers(A); *Taq* DNA polymerase recombinant (Invitrogen #11615-010); and treated Diethylpyrocarbonate (DEPC). Conditions at the Thermal Cycler T100™ (Bio Rad, #186-1096) were: 95ºC / 5min; 95ºC / 1min; 58ºC / 30 seconds; 72ºC / 1min; and 72ºC / 5min; repeated 50 times. Electrophoresis in Ultra Pure™ agarose gel (Invitrogen, #16500-100) with Ultra Pure™ ethidium bromide (Invitrogen, #15585011) was performed in duplicate on PowerPac™ Basic Power Supply (Bio Rad, #164-5050), with GeneRuler 1Kb Plus DNA Ladder (Thermo scientific, #SM1331). The amplified RT-PCR products were visualized on a Gel Doc™ EZ System.

Chi-square, Kruskal-Wallis, and descriptive data were used to compare the scores of intensity in positive cells. An association between EGFR and Her2 expression in normal prostatic tissues with PIA and PC was tested by Spearman test. It was used SPSS (IBM Corp. Released 2010. IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp.), and R (R Development Core Team (2008) R Foundation for Statistical Computing, Vienna). All values were ranked and considered with 5% significance level or less.

RESULTS

From the TMA slide, 15 (21.43%) samples of normal tissue, 30 (42.86%) of PIA, and 25 (35.71%) of PC were obtained and scored according to the intensity of immunostaining of EGFR and Her2 (Table 1). The immunostaining was present in the membrane going through cytoplasm (Figure 1). Regarding EGFR immunostaining intensity, there was no difference between the normal prostates and those with PIA (p=0.814) and PC (P=1.00) and PIA and PC (P=0.859). By contrast, there was difference in the intensity of Her2 immunostaining between normal tissues and those with PIA (P<0.001) and PC (P<0.001), as well as difference between samples with PIA and PC (P=0.018) (Table 1). When comparing EGFR and Her2 immunostaining's intensity, there was no correlation (r=0.055 and P=0.578).

From 70 samples, 16 cDNA samples of canine prostatic tissue with rates between A260/A280 higher than 1.70 were obtained through RT-PCR. There were four (25%) normal tissues, three (18.75%) with PIA, and nine (56.25%) with PC.

Table 1 - Means of comparison and labeling intensity scores of anti-EGFR and anti-Her2 antibodies in normal prostatic tissue and with canine proliferative inflammatory atrophy (PIA), and canine prostatic carcinoma (PC).

		Normal (n=15)	PIA (n=30)	PC (n=25)
----------EGFR----------				
Labeling intensity	0- Negative	20% (3)	6.67% (2)	24% (6)
	1+ (Mild)	53.33% (8)	56.67% (17)	44% (11)
	2+(Moderate)	26.67% (4)	33.33% (10)	28% (7)
	3+(Intense)	0% (0)	3.33% (1)	4% (1)
	Rank*	45.20[a]	55.86[a]	47.12[a]
----------Her2----------				
Labeling intensity	0- Negative	87.7% (13)	13.33% (4)	0% (0)
	1+(Mild)	13.3% (2)	26.67% (8)	16% (4)
	2+(Moderate)	0% (0)	23.33% (7)	12% (3)
	3+(Intense)	0% (0)	36.67% (11)	72% (18)
	Rank*	17.33[a]	56.66[b]	74.18[c]

*Result of Kruskal-Wallis test. Similar letters in the same line are not different (P>0.05).

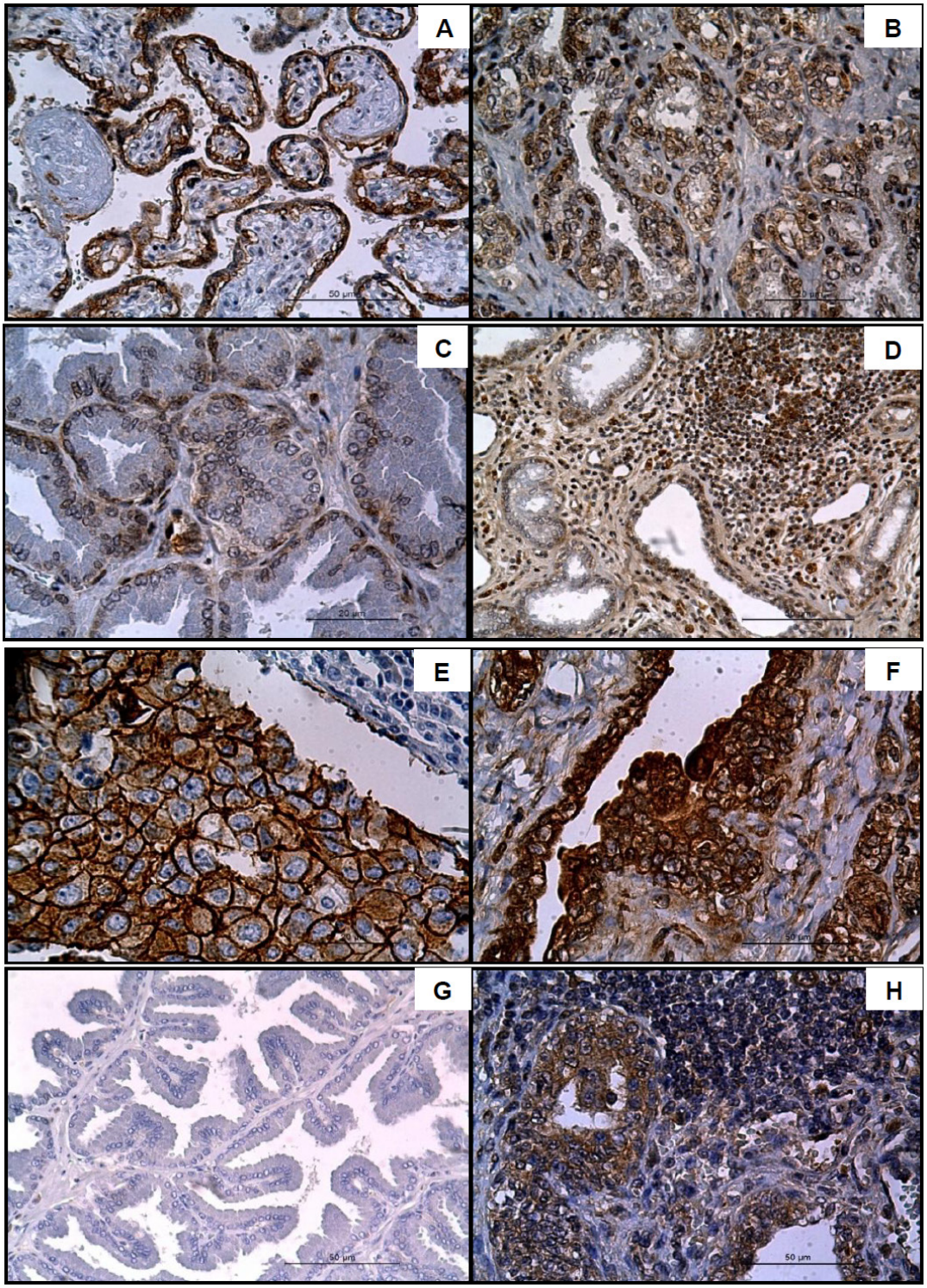

Figure 1 - A) EGFR in human placenta, positive control, 3+ score (200X); B) EGFR in canine PC, 1+score (400X); C) EGFR in normal canine prostatic tissue, 1+ score (400X); D) EGFR in canine PIA, 2+ score (200X). E) Her2 in human mammary carcinoma, positive control, 3+ score (400X); F) Her2 in canine PC, 3+score (400X); G) Her2 in normal canine prostatic tissue, 0- score (200X); H) Her2 in canine PIA, 2+ score (400X). IHC, DAB with hematoxylin counterstain.

The gene ErbB1 mRNA was detected in two normal tissues, one with PIA and in all PC samples. ErbB2 mRNA was detected in two samples of PIA (PIA2, PIA3), and in all PC, but it was not detected in normal prostatic tissue samples (Figure 2). A parallel of EGFR and Her2 immunolabeling by IHC and the detection of ErbB1 and ErbB2 through RT-PCR is shown in table 2.

DISCUSSION

EGFR immunostaining was observed in 80% of canine normal prostatic tissue. It was also detected ErbB1 mRNA in 50% of these tissues. The authors GAMA et al. (2009), PERALDO-NEIA et al. (2011), and CARLSSON et al. (2013), reported EGFR expression in normal tissues such as liver, digestive tract, human prostate, and canine mammary tissue. However, TERRAGNI et al. (2014) reported no expression of EGFR in canine normal gastric epithelium, similar to what was observed here in 20% of canine normal prostatic tissue.

In this study, 36.6% of the prostates with PIA and 32% with canine PC presented EGFR immunostaining. These results are similar to those obtained by CARLSSON et al. (2013) in their study with human PC and lymph node metastasis. They observed EGFR immunostaining in roughly 41% of primary tumors, and in one case of metastasis. By contrast, SCHLOMM et al. (2006) observed EGFR immunolabeling in 17.5% of human prostates with PC. Thus, CARLSSON et al. (2013) suggested that EGFR could have an important role in initial lesions of PC and it would be negligible in advanced-stage tumors.

Owing to the fact that in this study there was no difference in EGFR immunostaining between prostates with PIA and PC, it is possible that EGFR plays a role in premalignant and early lesions of canine prostate. Following that idea, DIPALMA et al. (2005) and MATSUBAYASHI & YOSHIHARA (2007) reported ErbB1 amplification and EGFR overexpression in early events of carcinogenesis in tumors of human salivary glands. Under these circumstances, BERTAGNOLLI et al. (2011) studied EGFR in canine mammary tumors and concluded that EGFR expression may contribute to malignant epithelial transformation. Those authors had also been reporting that EGFR is associated with increased tumor proliferative activity, angiogenesis and metastatic potential, working as a predictive factor in these neoplasms.

Her2 immunostaining was observed in two samples of canine normal prostatic tissues. The samples were scored 1+, and considered negative. These results are in agreement with TERRAGNI et al. (2014) who have not observed Her2 expression in no neoplastic canine gastric mucosa. However,

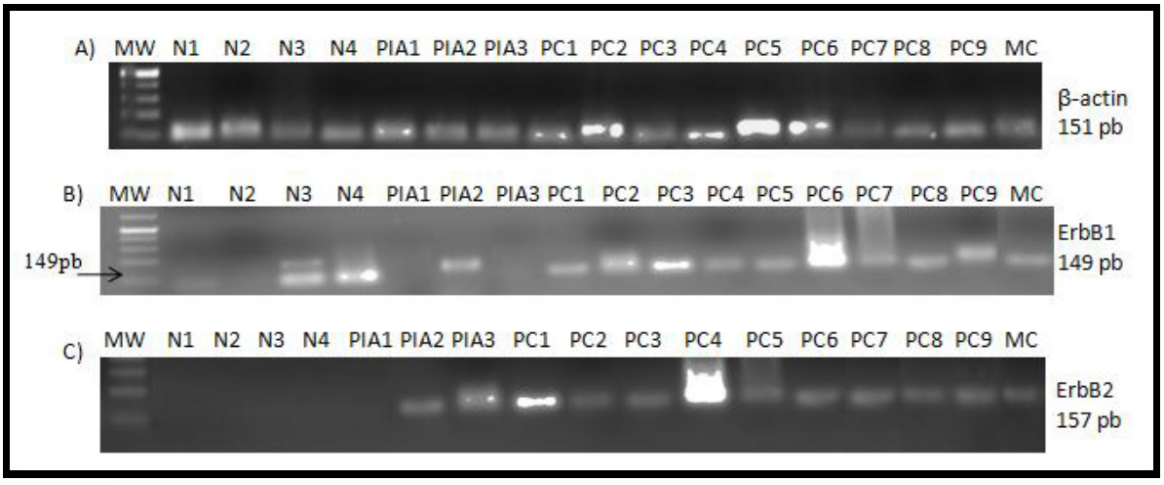

Figure 2 - Agarose gel images showing amplification products of β-actin, ErbB1, and ErbB2 genes from canine prostatic tissue. A) RT-PCR for β-actin expression as samples of positive control. All samples amplified. B) RT-PCR for ErbB1. Prostatic samples N3, N4, PIA2, all PC, and MC amplified. C) RT-PCR for ErbB2. Prostatic samples PIA2, PIA3, all PC and MC amplified. MW = Molecular weight (GeneRuler 1Kb Plus DNA Ladder - Thermo scientific, #SM1331); MC= canine mammary carcinoma (ErbB1 and ErbB2 positive control); N= canine normal prostatic tissue; PIA= canine proliferative inflammatory atrophy; PC= canine prostatic carcinoma.

Table 2 - Comparison results of anti-EGFR and anti-Her2 immunostaining scores by immunohistochemistry (IHC), and ErbB1 and ErbB2 detection by RT-PCR in normal canine prostatic tissue and with canine proliferative inflammatory atrophy (PIA), and canine prostatic carcinoma (PC).

Samples	----------IHC----------		----------RT-PCR----------	
	EGFR	Her2	ErbB1	ErbB2
N1	0 (-)	0 (-)	-	-
N2	1 (-)	0 (-)	-	-
N3	2 (+)	1 (-)	+	-
N4	1 (-)	0 (-)	+	-
PIA1	1 (-)	1 (-)	-	-
PIA2	2 (+)	2 (+)	+	+
PIA3	1 (-)	2 (+)	-	+
PC1	1 (-)	2 (+)	+	+
PC2	2 (+)	2 (+)	+	+
PC3	1 (-)	3 (+)	+	+
PC4	2 (+)	3 (+)	+	+
PC5	1 (-)	2 (+)	+	+
PC6	0 (-)	3 (+)	+	+
PC7	1 (0)	2 (+)	+	+
PC8	2 (+)	3 (+)	+	+
PC9	2 (+)	2 (+)	+	+
MC	3 (+)	3 (+)	+	+

MC=canine mammary carcinoma; N=canine normal prostatic tissue; PIA=canine proliferative inflammatory atrophy; PC=canine prostatic carcinoma; 0,1,2,3 IHC scores; + positive; - negative.

CARLSSON et al. (2013) reported that Her2 immunostaining may occur in normal tissues. Likewise, the IHC results concerning Her2 were in keeping with those obtained with RT-PCR technique, since there was no expression of ErbB2 in normal prostatic tissue.

Canine prostates with PC presented 84% of Her2 overlabeling and 100% ErbB2 expression. In human PC, Her2 overexpression is reported in 20-70% of PC samples (HERNES et al., 2004; BARTLETT et al., 2005) and it is associated with a high Gleason score, tumor recurrence, and advanced stages of cancer (SCHLOMM et al., 2006). However, LIU et al. (2001) reported no expression of Her2 in primary human PC. Conversely, CARLES et al. (2004) reported Her2 expression in up to 90% of PC cases, and CARLSSON et al. (2013), when studying human PC and lymph node metastasis, observed nearly 33% of Her2 immunostaining in primary tumors and 66.6% in metastasis. Those authors reported a higher frequency of Her2 overexpression in human PC cases with metastasis when compared to cases where PC was confined to the prostate, and they have suggested upregulation of Her2 in metastasis. On the basis of this, our results would indicate canine PC as having a great potential for aggressiveness as mentioned by LEROY &NORTHRUP (2009). However, even without evaluation for canine PC metastasis, it is likely that Her2 may have a single role in canine prostate tissue regarding PC evolution and metastasis, since canine PC is less frequent and has a later onset when compared with PC in humans. Perchance, mechanisms of Her2 interaction with inhibitory proteins involved in cell cycle, such as p21 and p27, are more efficient in canine than in human glands.

In this study, Her2 overlabeling and ErbB2 expression were reported in 60% and 66% of canine prostates with PIA, respectively, confirming the proliferative pattern of this prostatic disease and supporting its premalignant potential (De MARZO et al., 2006; LEROY &NORTHRUP 2009). TERRAGNI et al. (2014) observed that Her2 was more expressed in dysplastic areas of canine benign gastric tumors, suggesting its potential relevance in premalignant lesions. It is a relevant remark that Her2 immunostaining was significantly weaker in canine prostates with PIA than in those with PC. Likewise, ErbB2 mRNA was less detected in prostates with PIA than with PC. Even considering the small number

of samples amplified by PCR in this study, taken together, the results are suggestive that Her2 plays an important role in canine prostatic carcinogenesis and tumoral development.

CONCLUSION

EGFR and Her2 are expressed in canine PIA and early lesions of canine PC, particularly Her2, which is overexpressed, suggesting the involvement of these receptors in canine prostatic carcinogenesis and tumoral development. Considering these findings, the next step would be exploring the association of EGFR and Her2 in tumoral disease action, evolution, metastasis, and prognosis.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

The Comissão de Ética no Uso de Animais (CEUA) from Universidade Federal de Goiás (UFG), Goiânia, GO, approved this research (353/2010).

SOURCES AND MANUFACTURES

[A]Synthesized by Eurofins MWG Operon- EGFR Forward (Fw): CCAAGATCCCATCCATT - reverse (Rev): CTCGACAAGCTCTCTTT; Her2 Fw: AGTGCTGGATGATAGAC, Rev: AGTAGTGAACGGTAGAAG; β-actin, Fw: TGGAATCATGCGGTATC, Rev: GGCTGTGATTTCCTTCT. The primers were obtained from Primer Quest Advanced tool (www.idtdna.com).

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REFERENCES

BARTLETT, J.M.S. et al. Type I receptor tyrosine kinases are associated with hormone escape in prostate cancer. **Journal of Pathology**, v.205, n.4, p.522-529, 2005. Available from: <http://onlinelibrary.wiley.com/doi/10.1002/path.1735/abstract>. Accessed: Oct. 01, 2017. doi: 10.1002/path.1735.

BERTAGNOLLI, A.C. et al. Canine mammary mixed tumours: immunohistochemical expressions of EGFR and HER-2. **Australian Veterinary Journal,** v.89, n.8, p.312-317, 2011. Available from: <http://onlinelibrary.wiley.com/doi/10.1111/j.1751-0813.2011.00803.x/abstract>. Accessed: Oct. 01, 2017. doi: 10.1111/j.1751-0813.2011.00803.x.

BILOUS, M. et al. Current perspectives on HER2 testing: a review of national testing guidelines. **Modern Pathology**, v.16, n.2, p.173-182, 2003. Available from: <http://www.nature.com/modpathol/journal/v16/n2/full/3880738a.html?foxtrotcallback=true>. Accessed: Oct. 01, 2017. doi: 10.1097/01.MP.0000052102.90815.82.

CARLES, J. et al. Her-2/neu expression in prostate cancer: a dynamic process? **Clinical Cancer Research,** v.10, n.14, p.4742-4745, 2004. Available from: <http://clincancerres.aacrjournals.org/content/10/14/4742.long>. Accessed: Oct. 01, 2017. doi: 10.1158/1078-0432.CCR-04-0115.

CARLSSON, J. et al. Tendencies for higher co-expression of EGFR and HER2 and downregulation of HER3 in prostate cancer lymph node metastases compared with corresponding primary tumors. **Oncology Letters**, v.5, n.1, p.208-214. 2013. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3525498/>. Accessed: Oct. 01, 2017. doi: 10.3892/ol.2012.996.

CIARDELLO, F.; TORTORA, G. EGFR Antagonists in cancer treatment. **New England Journal of Medicine**, v.358, n.11, p.1160-1174, 2008. Available from: <http://www.nejm.org/doi/full/10.1056/NEJMra0707704>. Accessed: Oct. 01, 2017. doi: 10.1056/NEJMra0707704.

De MARZO, A.M. et al. A working group classification of focal prostate atrophy lesions. **American Journal of Surgical Pathology**, v.30, n.10, p.1281-1291, 2006. Available from: <http://journals.lww.com/ajsp/Abstract/2006/10000/A_Working_Group_Classification_of_Focal_Prostate.11.aspxp>. Accessed: Oct. 01, 2017. doi: 10.1097/01.pas.0000213289.50660.be.

Di PALMA, S. et al. Non-invasive (intracapsular) carcinoma ex pleomorphic adenoma: recognition of focal carcinoma by HER-2/neu and MIB1 immunohistochemistry. **Histopathology**, v.46, n.2, p.144-152, 2005. Available from: <http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2559.2005.02058.x/abstract>. Accessed: Oct. 01, 2017. doi: 10.1111/j.1365-2559.2005.02058.x.

GAMA, A. et al. Immunohistochemical expression of epidermal growth factor receptor (EGFR) in canine mammary tissues. **Research in Veterinary Science**, v.87, n.3, p.432-437, 2009. Available from: <http://www.sciencedirect.com/science/article/pii/S0034528809000988?via%3Dihub>. Accessed: Oct. 01, 2017. doi: 10.1016/j.rvsc.2009.04.016.

HERNES, E. et al. Expression of the epidermal growth factor receptor family in prostate carcinoma before and during androgen-independence. **British Journal of Cancer**, v.90, n.2, p.449-454, 2004. Available from: <https://www.nature.com/bjc/journal/v90/n2/full/6601536a.html>. Accessed: Oct. 01, 2017. doi: 10.1038/sj.bjc.6601536.

HSU, W.L. et al. Increased survival in dogs with malignant mammary tumours overexpressing HER-2 protein and detection of a silent single nucleotide polymorphism in the canine HER-2 gene. **Veterinary Journal**, v.180, n.1, p.116-123, 2009. Available from: <http://www.sciencedirect.com/science/article/pii/S1090023307003589?via%3Dihub>. Accessed: Oct. 01, 2017. doi: 10.1016/j.tvjl.2007.10.013.

LAI, C.L. et al. Histopathological and immunohistochemical characterization of canine prostate cancer. **Prostate**, v.68, n.5, p.477-488, 2008. Available from: <http://onlinelibrary.wiley.com/doi/10.1002/pros.20720/abstract>. Accessed: Oct. 01, 2017. doi: 10.1002/pros.20720.

LEROY, B.E.; NORTHRUP, N. Prostate cancer in dogs: comparative and clinical aspects. **Veterinary Journal**, v.180, n.2, p.149-162, 2009. Available from: <http://www.sciencedirect.com/science/article/pii/S1090023308002670>. Accessed: Oct. 01, 2017. doi: 10.1016/j.tvjl.2008.07.012.

LIU, H.L. et al. Detection of low level HER-2/neu gene amplification in prostate cancer by fluorescence in situ hybridization. **Cancer**

Journal, v.7, n.5, p.395-403, 2001. Available from: <https://www.scopus.com/record/display.uri?eid=2-s2.0-0035464538&origin=inward&txGid=0be00b1d5ad84afe232895eb9ad104b0#>. Accessed: Oct. 01, 2017.

MATSUBAYASHI, S.; YOSHIHARA, T. Carcinoma ex pleomorphic adenoma of the salivary gland: an immunohistochemical study. **European Archives of Otorhinolaryngology**, v.264, n.7, p.789-795, 2007. Available from: <https://link.springer.com/article/10.1007/s00405-007-0256-6>. Accessed: Oct. 01, 2017. doi: 10.1007/s00405-007-0256-6.

PERALDO-NEIA, C. et al. Epidermal growth factor receptor (EGFR) mutation analysis, gene expression profiling and EGFR protein expression in primary prostate cancer. **BMC Cancer**, v.11, n.31, p.1-12, 2011. Available from: <https://bmccancer.biomedcentral.com/articles/10.1186/1471-2407-11-31>. Accessed: Oct. 01, 2017. doi: 10.1186/1471-2407-11-31.

PUTZI M.J.; DE MARZO A.M. Morphologic transitions between proliferative inflammatory atrophy and high-grade prostatic intraepithelial neoplasia. **Urology**. v.56, n.5, p.828-832, 2000. Available from: <http://www.goldjournal.net/article/S0090-4295(00)00776-7/fulltext>. Accessed: Oct. 01, 2017. doi: 10.1016/S0090-4295(00)00776-7.

RESSEL, L. et al. HER-2 expression in canine morphologically normal, hyperplastic and neoplastic mammary tissues and its correlation with the clinical outcome. **Research in Veterinary Science**, v.94, n.2, p.299-305, 2013. Available from: <http://www.sciencedirect.com/science/article/pii/S0034528812002949>. Accessed: Oct. 01, 2017. doi: 10.1016/j.rvsc.2012.09.016.

RUBIN, M.A. et al. Tissue microarray sampling strategy for prostate cancer biomarker analysis. **American Journal of Surgical Pathology**, v.26, n.3, p.312-319, 2002. Available from: <http://journals.lww.com/ajsp/Abstract/2002/03000/Tissue_Microarray_Sampling_Strategy_for_Prostate.4.aspx>. Accessed: Oct. 01, 2017. Accession: 00000478-200203000-00004.

SABATTINI, S. et al. EGFR overexpression in canine primary lung cancer: pathogenetic implications and impact on survival. **Veterinary and Comparative Oncology**, v.12, n.3, p.237-248, 2014. Available from: <http://onlinelibrary.wiley.com/doi/10.1111/vco.12002/abstract>. Accessed: Oct. 01, 2017. doi: 10.1111/vco.12002.

SCHLOMM, T. et al. Epidermal growth factor receptor family members (EGFR and HER2) are prognostic markers and potential therapeutic targets in prostate cancer. **European Urology Supplement**, v.5, n.2, p.161, 2006. Available from: <http://www.sciencedirect.com/science/article/pii/S1569905606605599>. Accessed: Oct. 01, 2017. doi: 10.1016/S1569-9056(06)60559-9.

SHIOMITSU, K. et al. Expression of epidermal growth factor receptor and vascular endothelial growth factor in malignant canine epithelial nasal tumors. **Veterinary and Comparative Oncology**, v.7, n.2, p.106-114, 2009. Available from: <http://onlinelibrary.wiley.com/doi/10.1111/j.1476-5829.2008.00178.x/abstract>. Accessed: Oct. 01, 2017. doi: 10.1111/j.1476-5829.2008.00178.x.

TERRAGNI, R. et al. EGFR, HER-2 and KRAS in canine gastric epithelial tumors: A potential human model? **Plos One**, v.9, n.1:e85388, 2014. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3893207/>. Accessed: Oct. 01, 2017. doi: 10.1371/journal.pone.0085388.

TOLEDO, D.C. et al. Histomorphological characterization of proliferative inflammatory atrophy in canine prostate. **Ciência Rural**, v.40, n.6, p.1372-1377, 2010. Available from: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0103-84782010000600021>. Accessed: Oct. 01, 2017. doi: 10.1590/S0103-84782010000600021.

YARDEN, Y., SLIWKOWSKI, M.X. Untangling the ErbB signaling network. **Nature Reviews Molecular Cell Biology**, v.2, n.2, p.127-137, 2001. Available from: <http://www.nature.com/nrm/journal/v2/n2/full/nrm0201_127a.html>. Accessed: Oct. 01, 2017. doi: 10.1038/35052073.

WANG, W. et al. Morphological transition of proliferative inflammatory atrophy to high-grade intraepithelial neoplasia and cancer in human prostate. **Prostate**. v.69, n.13, p.1378-1386, 2009. Available from: <http://onlinelibrary.wiley.com/doi/10.1002/pros.20992/abstract>. Accessed: Oct. 01, 2017. doi: 10.1002/pros.20992.