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Immunohistochemical evaluation of HER2 expression in canine thyroid carcinoma

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ABSTRACT

The human epidermal growth factor receptor 2 (HER2) is expressed in various human cancers including thyroid cancers (TC) and is used as a diagnostic marker and therapeutic target. Canine TC (cTC), the most common endocrine malignancy in dogs, shows a high metastasis rate, and HER2-targeted therapy could be a candidate for treatment. Here, we immunohistochemically evaluated HER2 expression in 21 paraffin-embedded cTC tissues and scored the degree of expression based on intensity and positivity (score: 0–3+). Four samples (19%) scored 3+; 6 (29%), 2+; 7 (33%), 1+; and 4 (19%), 0. Therefore, 48% of the cTC tissues were HER2 positive (scored ≥2+). These data may lead to further evaluation of the role of HER2 in cTC as a mechanism of malignancy and a therapeutic target.

1. Introduction

Human epidermal growth factor receptor 2 (HER2) belongs to the epidermal growth factor receptor (EGFR) family and is expressed in various human cancers including breast, gastric, and thyroid cancers (TC) (Dai et al., 2017; English et al., 2013; Ménard et al., 2001). Because HER2 overexpression is related to carcinogenesis and aggressive phenotype in these cancers, HER2 is used as a diagnostic marker and therapeutic target (English et al., 2013). In veterinary medicine, HER2 expression was reported in canine mammary gland tumours, osteosarcoma, and transitional cell carcinomas (Gama et al., 2008; Mason et al., 2016; K. Sakai et al., 2018). Moreover, HER2-targeted therapies like lapatinib and recombinant *Listeria* vaccines show an anti-tumour effect on canine HER2-positive cancers (Mason et al., 2016; Sakai et al., 2018).

Canine TC (cTC) is the most common endocrine malignancy in dogs and shows a high metastasis rate (Liptak, 2007). Although additional systemic therapies are required for dogs with cTC, general chemotherapy protocol demonstrated little effect on survival rates (Nadeau and Kitchell, 2011). Thus, novel systemic therapies are needed. While HER2 is overexpressed in human TC, HER2 expression in cTC has not been evaluated. As HER2-targeted therapy could be an anti-cancer systemic therapy for dogs, we evaluated HER2 expression in cTC via immunohistochemical analysis.

2. Materials and methods

Paraffin-embedded cTC tissues and medical records were retrospectively evaluated with permission from the owners. These tissues were surgically removed at the Veterinary Medical Center of The University of Tokyo between January 2012 and December 2016. Veterinary pathologists certified by the Japanese College of Veterinary Pathologists diagnosed the samples. All patients were clinically staged by an ultrasound examination of the neck, thoracic radiography, and computed tomography (Liptak, 2007). HER2 expression of all samples were immunohistochemically evaluated (Wolff et al., 2013). Antigen retrieval was conducted using Dako Target Retrieval Solution, pH 9.0 (Agilent Technologies, Santa Clara, CA, USA) by microwaving for 15 min at 750 W. After endogenous peroxidase was blocked, specimens were incubated in 8% skimmed milk for 1 h at 20–28 °C with mouse IgG1 anti-HER2 monoclonal antibodies, frequently used for staining canine HER2

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Table 1
Characteristics of dogs and the corresponding HER2 expression.

No	Breed[a]	Age (years)	Sex[b]	Location	TNM classification			Clinical stage	IHC score
					T	N	M		
1	MD	12.1	M	Ectopic	T1	N0	M0	1	2+
2	CH	11.6	FS	Right	T2	N0	M0	2	0
3	SCHI	13.1	MC	Left	T2	N0	M0	2	0
4	SHI	6.8	M	Left	T2	N0	M0	2	1+
5	BG	9.2	MC	Bilateral	T2	N0	M0	2	1+
6	BG	7.9	M	Bilateral	T2	N0	M0	2	1+
7	YT	12.0	MC	Ectopic	T2	N0	M0	2	2+
8	WCP	12.7	MC	Bilateral	T2	N0	M0	2	2+
9	LR	11.3	MC	Right	T2	N0	M0	2	2+
10	LR	7.3	FS	Right	T2	N0	M0	2	3+
11	MD	11.9	MC	Right	T2	N0	M0	2	3+
12	MIX	11.5	FS	Right	T2	N0	M0	2	3+
13	MD	8.3	FS	Right	T2	N0	M0	2	3+
14	PE	7.1	M	Bilateral	T2	N2	M0	2	2+
15	MIX	11.0	M	Right	T3	N0	M0	3	0
16	MD	6.5	FS	Right	T3	N0	M0	3	1+
17	WCP	10.7	MC	Left	T3	N0	M0	3	1+
18	YT	11.9	M	Bilateral	T3	N1	M0	3	0
19	BG	10.4	M	Right	T3	N1	M0	3	1+
20	MIX	13.8	MC	Right	T2	N0	M1	4	1+
21	BG	8.5	M	Bilateral	T3	N0	M1	4	2+

[a] MD: Miniature Dachshund, CH: Chihuahua, SCHI: Schipperke, SHI: Shih Tzu, BG: Beagle, YT: Yorkshire Terrier, WCP: Welsh Corgi Pembroke, LR: Labrador Retriever, PE: Pekingese.
[b] M: Male, MC: Male castrated, F: Female, FS: Female spayed.

(1:40, clone: CB-11, Leica biosystems, Wetzlar, Germany) (Gama et al., 2008) or with mouse IgG1, κ isotype antibody (Clone: MG1-45, BioLegend, San Diego, CA, USA) as a negative control at 4 °C overnight. Sections were then incubated with a horseradish peroxidase (HRP)-conjugated anti-mouse antibody (Envision + System-HRP Labelled Polymer; K4001; Agilent Technologies) and 3, 3′-diaminobenzidine (Dojindo Laboratories, Rockville, MD, USA) solution. The results were quantified per the American Society of Clinical Oncology/College of American Pathologists guidelines (ASCO/CAP) (Wolff et al., 2013).

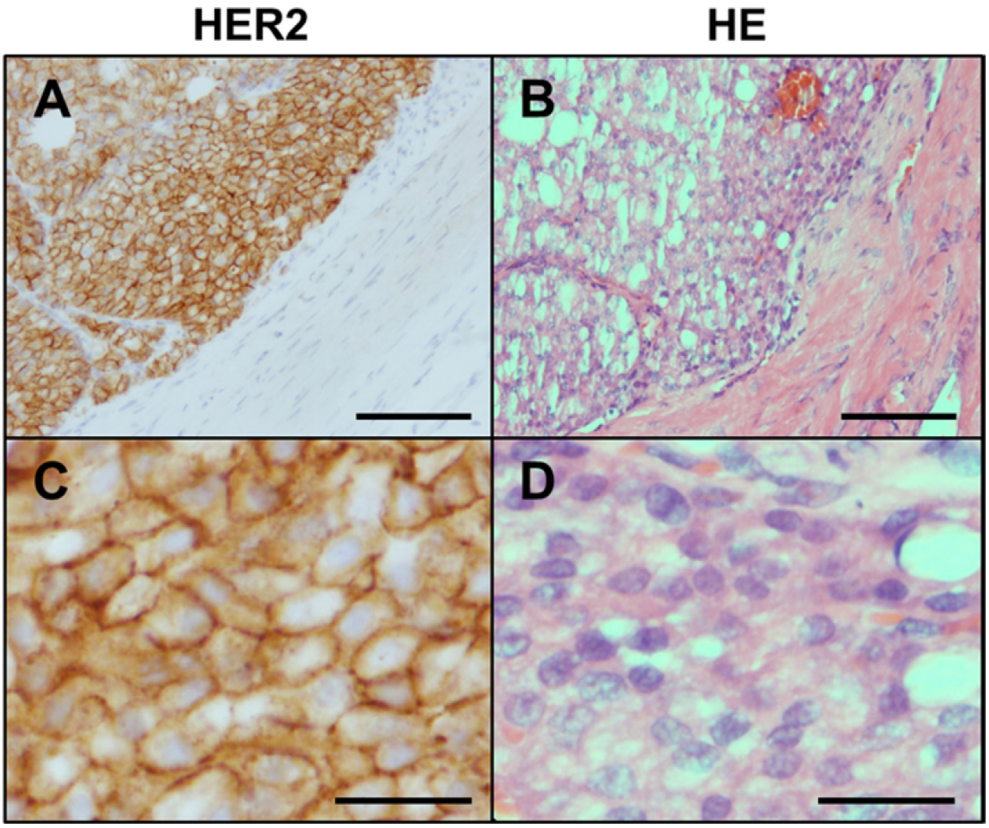

Fig. 1. Representative staining pattern of HER2 in cTC. (A, C) Circumferential membrane staining is complete and intense in cTC. (B, D) HE staining of cTC. (A, B) Scale bar, 100 μm (C, D) Scale bar, 25 μm.

Table 2
HER2 scoring methods proposed by ASCO/CAP.

Score	Description
3+	Circumferential membrane staining that is complete, intense, and in >10% of tumor cells
2+	Circumferential membrane staining that is incomplete and/or weak/moderate and in >10% of tumor cells or Complete and circumferential membrane staining that is intense and in <10% of tumor cells
1+	Incomplete membrane staining that is faint/barely perceptible and in >10% of tumor cells
0	No staining is observed or Membrane staining that is incomplete and is faint/barely perceptible and in <10% of tumor cells.

3. Results

Twenty-one TC tissue samples were collected from 21 dogs (8 castrated males, 8 intact males, 5 spayed females) of different breeds (4 Beagles, 4 Miniature Dachshunds, 3 mixed breeds, 2 Labrador Retrievers, 2 Welsh Corgi Pembrokes, 2 Yorkshire Terriers, 1 Chihuahua, Pekinese, Schipperke, and Shih Tzu). The mean age was 10.3 ± 2.3 years (population standard deviation), ranging from 6.5-13.8 years. Thirteen (62%) tumours were unilateral; 6 (29%), bilateral; and 2 (10%), ectopic. One case had stage 1 disease, and 13 (62%), 5 (24%), and 2 (10%) cases had stage 2, 3, and 4, respectively (Table 1).

HER2-positive cTC tissues demonstrated strong broad staining of the circumferential membrane of cTC cells and negative staining on peritumour normal cells via immunohistochemical staining (Fig. 1). We analysed the HER2 expression score based on ASCO/CAP (Table 2) to evaluate the staining pattern. Representative staining patterns of each score are shown in Fig. 2. HER2 expression score analysis demonstrated that 4 samples (19%) scored 3+; 6 samples (29%), 2+; 7 samples (33%), 1+; and 4 samples (19%), 0 (Table 3). Almost half (48%) of the cTC tissues were HER2 positive (scored 2+ <). Statistical analysis revealed that there was no relationship between HER2 score and clinical data such as location, TNM classification and clinical stage (Table 1).

4. Discussion

We confirmed circumferential membrane staining of canine HER2 in cTC. HER2 expression was strongly detected in 48% of the cTC cells. In human TC, HER2 expression was high with aggressive behaviours including extrathyroidal extension, lymph node metastasis, and high TNM stage (Dai et al., 2017). Thus, we statistically evaluated association of HER2 expression with clinical data in our samples but observed no correlation between HER2 expression and clinical characteristics such as location, TNM classification and clinical stage. Because we evaluated a limited number of patients, further studies are required to investigate the association between HER2 expression and malignancies in cTC.

Table 3
HER2 scores in cTC tissue samples.

	Negative		Positive	
Score	0	1+	2+	3+
Number (%)	4 (19%)	7 (33%)	6 (29%)	4 (19%)

Multiple HER2-targeting drugs including cytotoxic antibodies, molecular-targeted drugs, and vaccines have shown clinical benefits in humans and canines (English et al., 2013). We demonstrated that half of the dogs with cTC can potentially benefit from HER2-targeting therapy though the number of cases were limited. Because canine and human HER2 have 92% amino acid homology (Singer et al., 2012), some of the human HER2-targeting drugs could have a therapeutic effect on dogs. Although further studies on HER2 downstream signalling in cTC are required, lapatinib, which has an anti-tumour effect in canine cancers in laboratory studies (Sakai et al., 2018), is an effective candidate for a HER2-positive cTC drug. Mason et al. reported a potential anti-tumour effect of recombinant *Listeria*-HER2 vaccine on dogs with osteosarcoma (Mason et al., 2016). Because vaccine therapy is independent for HER2 downstream signalling, the recombinant *Listeria*-HER2 vaccine would have an anti-tumour effect in a broad spectrum of dogs with HER2-positive cTC as well as canine osteosarcoma. Our preliminary data lead to further evaluation of the role of HER2 in cTC as a marker of malignancy and a therapeutic target for HER2-targeted therapies.

Declarations

Author contribution statement

Sho Yoshimoto: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Daiki Kato, Takayuki Nakagawa: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Satoshi Kamoto, Masahiro Shinada, Namiko Ikeda:Performed the experiments.

Kie Yamamoto; Performed the experiments; Analyzed and interpreted the data.

Masaya Tsuboi, Kohei Saeki: Analyzed and interpreted the data.

Yuiko Tanaka: Analyzed and interpreted the data; Wrote the paper.

Ryohei Yoshitake, mShotaro Eto, James Chambers, Ryohei Kinoshita, Kazuyuki Uchida: Contributed reagents, materials, analysis tools or data.

Ryohei Nishimura: Conceived and designed the experiments; Wrote the paper.

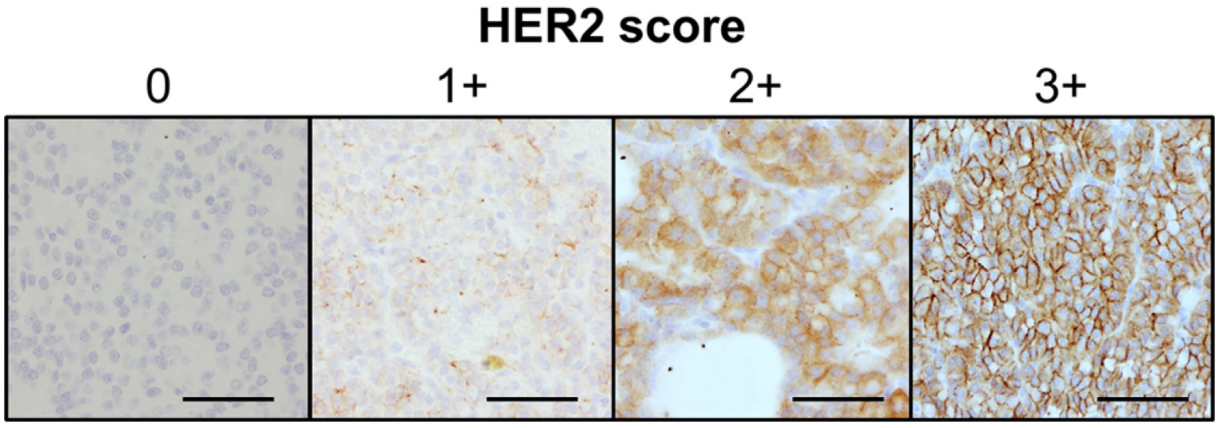

Fig. 2. HER2 scoring of canine TC based on ASCO/CAP. Representative images of the staining pattern of HER2 in canine TC. Scale bar, 100 μm.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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