Original Article

Selection of Reference Genes for Real-time Polymerase Chain Reaction in Canine Oral Tumor and Cancer

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Abstract

Tumors in oral cavity either benign or malignant are often found in dogs. Despite the increasing number of studies of gene expression associated with canine oral cavity diseases, reference gene selection for normalizing target genes has not been performed. The objective of the present study was to identify potential reference genes in canine oral tumors, including epulis, ameloblastoma, oral melanoma, oral squamous cell carcinoma, etc. Suitability of 6 candidate reference genes, beta-actin (*ACTB*), beta-2-microglobulin (*B2M*), glyceraldehydes-3-phosphate dehydrogenase (*GAPDH*), ribosomal subunit L13a (*RPL13a*), ribosomal protein S5 (*RPS5*) and ribosomal protein S19 (*RPS19*), was evaluated with 5 algorithms, geNorm, Normfinder, BestKeeper, the comparative ΔCt method and RefFinder. Except Bestkeeper, most algorithms showed that a cohort of *RPS5, RPS19* and *ACTB* was most suitable to normalize target genes. Since some algorithms did not show the same ranks of reference genes, it is necessary to normalize target genes against more than 1 reference gene with more than 1 algorithm. In conclusion, this work suggests that *ACTB* in combination with *RPS19* and *RPS5* are validated to be used as reference genes for expression study in canine oral tumors. These reference genes will increase the reliability of any qRT-PCR gene expression analysis of canine oral tumor and cancer in several aspects such as clinical diagnosis, prognosis and drug testing.

Keywords: canine, oral cancers, oral tumors, real-time RT-PCR, reference gene

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Introduction

Head and neck neoplasms account for approximately 7% of all canine tumors (Brønden et al., 2009). Two of the most common head and neck cancers (HNC) are oral squamous cell carcinoma (OSCC) and oral melanoma (OM) (Brønden et al., 2009; Priester and McKay, 1980). Oral benign and malignant tumors display different behaviors. The benign tumors such as epulis and ameloblastoma do not metastasize and complete excision is curative, whereas the malignant tumors are locally invasive such as fibrosarcoma and some tend to spread to other organs such as OSCC, OM and osteosarcoma. For OSCC, the metastatic rate depends on the location of the primary tumor: the rostral mouth (non-tonsillar OSCC) has a low metastatic rate while those in the caudal tongue and tonsils (tonsillar OSCC) have a high metastatic risk (MacEwen, 1990). Approximately 44% and 31% of all oral malignancies in dogs are oral melanoma (OM) and oral squamous cell carcinoma (OSCC), respectively (Priester and McKay, 1980). The World Health Organization (WHO) has defined a clinical staging scheme for dogs with OM based on tumor size and metastasis as stages I-IV from low to high virulence (Bergman, 2007). For OSCC, histological grading is widely used to help prognosis and grades I-III refer to well, moderately and poorly differentiated, respectively (Nemec et al., 2012). Since the gene expression profiles of canine melanoma are usually demonstrated by quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR), normalization to reference genes is required to remove any technical variation during the qRT-PCR processes (Radonić et al., 2004). According to the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines, the optimal number of reference genes must be experimentally determined and the statistical algorithms used to identify the best reference genes have to be reported (Bustin et al., 2009). Several publications of reference genes were addressed in animals such as pigs (Park et al., 2015), bovine (Spalenza et al., 2011), horses (Hjertner et al., 2013), chicken (Bagés et al., 2015), and cats (Jursza et al., 2014). In dogs, a number of reference genes were commonly used in gene expression study, including β-actin (*ACTB*) (Peleg et al., 2010), β-2-microglobulin (*B2M*) (Brinkhof et al., 2006), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) (Park et al., 2013), ribosomal subunit L13a (*RPL13a*) (Peters et al., 2007), ribosomal protein S5 (*RPS5*) and ribosomal protein S19 (*RPS19*) (Schlotter et al., 2009; Theerawatanasirikul et al., 2012). However, only few publications of potential reference genes in canine cancers have been reported (Etschmann et al., 2006; Tsai and Breen, 2012; Zornhagen et al., 2014), excluding canine oral cancers.

The objectives of the present study were to identify suitable reference genes in canine normal gingival tissues, cancerous and non-cancerous oral tumors, and to compare different algorithms in terms of expression stability. The expression stability of six potential reference genes was assessed, including *ACTB*, *GAPDH*, *B2M*, *RPL13a*, *RPS5* and *RPS19*, using five statistical algorithms, geNorm (Vandesompele et al., 2002), NormFinder (Andersen et al., 2004), BestKeeper (Pfaffl et al., 2004), comparative delta threshold cycle (dCt) (Silver et al., 2006), and RefFinder (Taki et al., 2014).

Materials and Methods

Animals: Twelve OM tissues, 9 non-tonsillar OSCC, 8 other oral cancers and 9 benign oral tumors were obtained from dogs submitted to surgery at the Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University (age range: 10-16 years). Eight normal gingival samples were collected from dogs with no history and clinical signs of oral cavity or cancerous problems (age range: 8 months-13 years). The affected dogs were measured for tumor size, lymph node examined, and thoracic and abdominal radiograph (TNM). Clinical staging of the affected dogs was done according to TNM staging. The samples were immediately collected after anesthesia and from freshly necropsied dogs submitted to the Department of Veterinary Pathology, Faculty of Veterinary Science, Chulalongkorn University (Thailand). The samples were obtained with the consent of owners following the ethical guidelines required under the Chulalongkorn University Animal Care and Use Committee (CU-ACUC), Thailand (Protocol No. 1531005).

Isolation of samples: For the OM samples, Melan-A immunohistochemical staining was performed to confirm amelanotic melanoma diagnosis, as described below. Melanoma cases were histopathologically diagnosed based on cell types; melanotic or amelanotic, epithelioid or spindle (Table 1). The OSCC samples were histopathologically classified into 6 cases of well and 3 cases of poorly differentiated. Other oral cancers included 2 fibrosarcoma, 2 hemangiosarcoma, 1 osteosarcoma, 1 basal cell carcinoma, 1 malignant peripheral nerve sheath tumor and 1 malignant schwanoma with osteosarcoma. The benign tumors composed of 6 epulis and 3 ameloblastoma (Fig. 1).

Table 1 Clinical stages and histological types of OM samples used in the present study

Melanotic (M)/ Amelanotic (A)	TNM Stage 1*	TNM Stage 2	TNM Stage 3	TNM Stage 4	Total
M	1		3	1	5
A		2**	5		7
Total	1/12 8.33%	2/12 16.67%	8/12 66.67%	1/12 8.33%	12 100%

*TNM stage I = < 2 cm diameter tumor, stage II = 2 cm to < 4 cm diameter tumor, stage III = 4 cm or greater tumor and/or lymph node metastasis, and stage IV = distant metastasis

**Most dogs had epitheloid cell type except one dog in TNM stage 2 had spindle cell type.

Table 2 Primers used in the present study.

Gene	Accession number	Primers (5'-3')	Length (bp)	Tm* (ºC)
β-actin (*ACTB*)	AF021873.2	Fwd 5'-ATGGAATCATGCGGTATCCAC-3' Rev 5'-CTTCTGCATCCTGTCAGCAA-3'	141	60.38 58.54
β -2 microglobulin (*B2M*)	XM_003640047.2	Fwd 5'-TCCCCCAAAGATTCAAGTGT-3' Rev 5'-ATGGAACCCTGACACGTAGC-3'	85	57.86 58.46
Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*)	NM_001003142.1	Fwd 5'-GCCCTCAATGACCACTTTGT-3' Rev 5'-TCCTTGGAGGCCATGTAGAC-3'	101	58.43 58.53
Ribosomal protein L13a (*RPL13a*)	XM_003432726.2	Fwd 5'-ATCCCACCACCCTATGACAA-3' Rev 5'-TCCTCCAGGGTTGCTGTTAC-3'	152	58.50 58.58
Ribosomal protein S5 (*RPS5*)	XM_533568.4	Fwd 5'-TCACTGGTGAGAACCCCCT-3' Rev 5'-CCTGATTCACACGGCGTAG-3'	141	58.89 58.68
Ribosomal protein S19 (*RPS19*)	XM_003639381.2	Fwd 5'-CCTTCCTCAAAAAGTCTGGG-3' Rev 5'-GTTCTCATCGTAGGGAGCAAG-3'	95	57.28 57.46
E-cadherin (*CDH1*)	XM_536807.3	Fwd 5'-GGTGCTCACATTTCCCAGTT-3' Rev 5'-AAATGGGCCTTTCTCGTTTT-3'	100	58.43 58.54

*Tm calculator by http://www6.appliedbiosystems.com/support/techtools/calc/

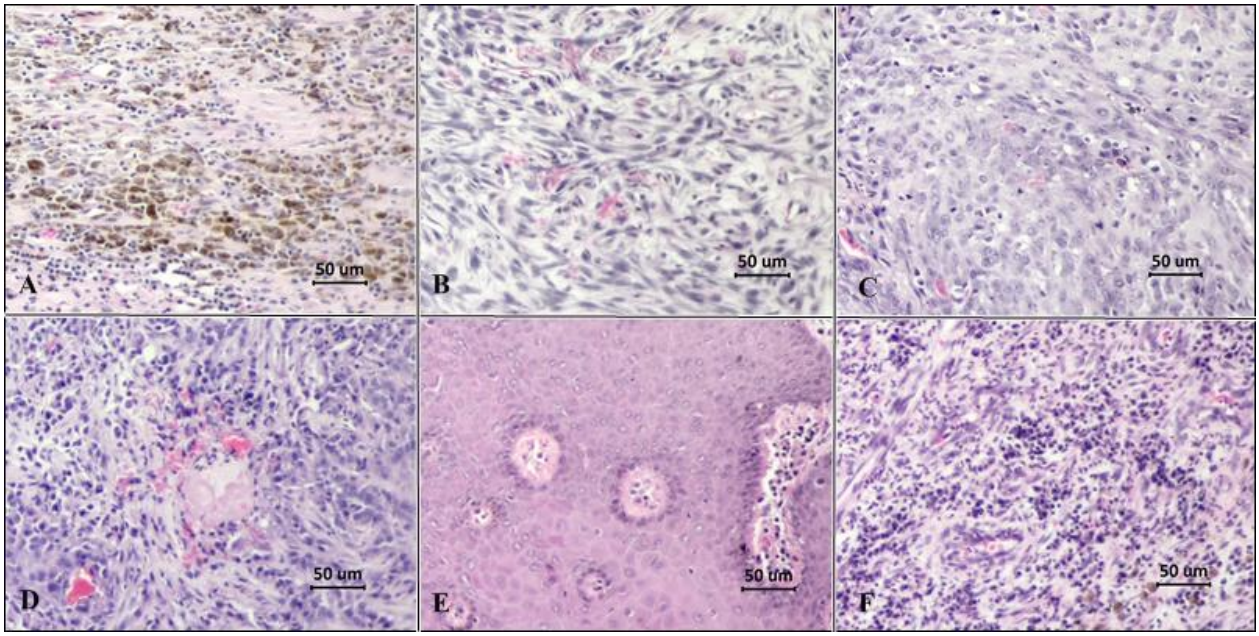

Figure 1 Histopathology of melanotic melanoma (A), amelanotic melanoma (B), well-differentiated squamous cell carcinoma (C), poor-differentiated squamous cell carcinoma (D), epulis (E) and ameloblastoma (F) (scale bar = 50 µm)

The tissues were divided for histopathological and immunohistochemical (IHC) study and for qRT-PCR study. For the histopathological and IHC study, the samples were immersed in 10% neutral buffered formalin for 24 h, followed by standard tissue processing and paraffin embedding. For the qRT-PCR, the samples were kept in RNALater solution overnight at 4˚C and stored at -20˚C until processed.

Histopathology and immunohistochemistry: The tumor samples in paraffin-embedded blocks were cut into sections (4-micron thickness) and mounted on glass slides. The slides were deparaffinized in xylene, rehydrated in graded ethanol, and then stained with hematoxylin and eosin (H&E) for routine histopathological evaluation. Immunohistochemistry was used to confirm the diagnosis of amelanotic melanoma with a mouse monoclonal antibody against human Melan-A (M7196, Dako, Glostrup, Denmark). Antigen retrieval was performed by a microwave oven in 0.01 M sodium citrate, pH 6.0, at 800 W for 10 min. The slides were incubated with anti-Melan-A antibody at dilution of 1:50 at 4˚C for 12 h. Primary antibody binding was detected by a polymer-based non avidin-biotin system, EnVision detection system (Dako, Glostrup, Denmark) and visualized with a 3,3´-diaminobenzidine tetrahydrochloride (DAB) substrate kit (Dako, Glostrup, Denmark). The slides were counterstained with Mayer's hematoxylin. A positive control was a canine oral melanotic melanoma section. Staining results were evaluated to positively or negatively cytoplasmic area (Fig. 2).

RNA isolation: Total RNA was extracted using a Nucleospin RNA kit (Macherey-Nagel, Dueren, Germany) according to the manufacturer's protocol. The total RNA samples were treated twice with TURBO DNase (Thermo Fisher Scientific/Life Technologies, Grand Island, NY) for 30 min at 37°C each round to remove contaminating genomic DNA and pseudogenes. Concentration of the RNA was determined using a NanoDrop ND-1000 Spectrophotometer V3.7 (Thermo Fisher Scientific, Waltham, MA) by measuring absorbance at a wavelength of 260 nanometre (A_{260}); the A_{260}nm/A_{280} nm ratio reflected RNA purity ranging from 1.8 to 2.2. Integrity of the RNA was determined by 1% agarose gel electrophoresis to assess the 28S and 18S bands.

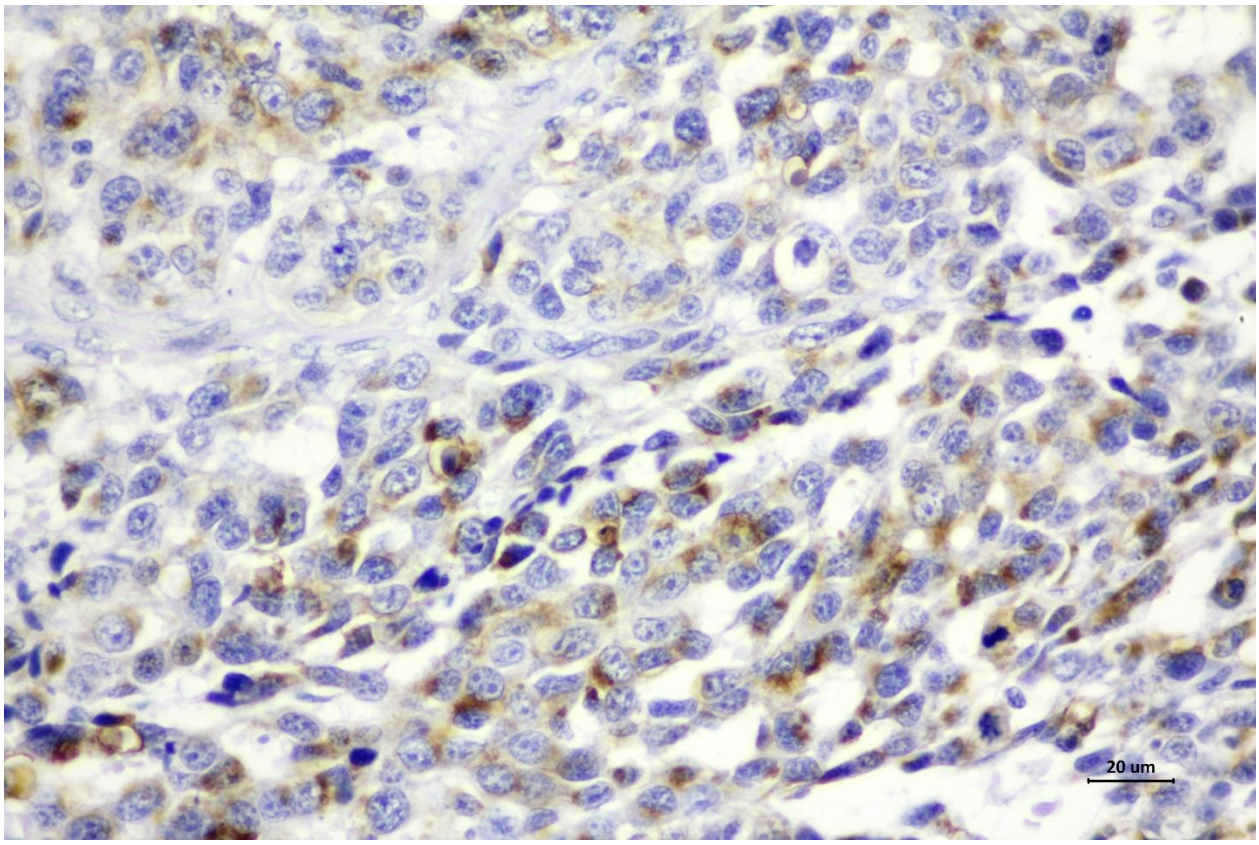

Figure 2 Immunohistochemical staining for Melan-A in amelanotic melanoma (scale bar = 20 µm)

Primer design and testing: Primer sequences of *RPS5* and *RPS19* have been previously described (Schlotter et al., 2009). Other primers were developed using Primer 3 software (Rozen and Skaletzky, 1999). Specificity of each primer was verified using the In-Silico PCR web application (http://genome.ucsc.edu/cgi-bin/hgPcr), a virtual PCR against canine reference genomes (CanFam 3.1, September 2011 assembly), and the Basic Local Alignment Search Tool (BLAST; http://www.ncbi.nlm.nih.gov/blast), returning Genbank accession numbers. DNA sequencing was performed to verify the genes. The primer sequences, accession numbers, and amplicons are depicted as a supplementary file (Table 2).

Quantitative reverse transcription PCR: The DNase-treated RNA was converted to cDNA using the SuperScript III First strand synthesis system for RT-PCR (Life Technologies, Carlsbad, CA) according to the manufacturer's instructions. Briefly, one microgram of RNA was reverse transcribed in a 20 µL reaction containing 50 ng random primers, 40 U RNase inhibitor and 200 U Superscript III enzyme. qRT-PCR was performed by SYBR Green chemistry (KAPA SYBR Fast qPCR Master Mix Universal; KAPA Biosystems, Cambridge, MA) and analyzed on Rotor Gene 3000 Thermal Cycler (Qiagen, Hilden, Germany). PCR reactions were performed as previously described (Theerawatanasirikul et al., 2012). Primers were used at 200 nM each and cDNA at 25 ng in 10 µL reactions. Thermal cycling conditions were performed according to the manufacturer's instructions: 95°C for 2 min followed by 40 cycles at 95°C for 3 s, 60°C for 20 s and 72°C for 1 s. Each reaction was performed in duplicate in three independent runs. Only Cq from samples with duplicate Cq difference < 1 was further analyzed. A melting curve from 72°C with a rate of 1°C per second up to 95°C was analyzed to verify purity of the PCR products. Real-time data analysis was performed by REST-2009 (Relative Expression Software Tool) software (Pfaffl et al., 2002) with a detection threshold at 0.1. Real-time PCR efficiencies of 87.8-112.1% were acquired from standard curves of the fluorescent data from a standardized dilution series of cDNA from the same normal dog.

Statistical and expression stability analyses: Five algorithms were used to rank the reference genes according to their expression stability, including geNorm (Vandesompele et al., 2002), NormFinder

(Andersen et al., 2004), BestKeeper (Pfaffl et al., 2004), dCt (Silver et al., 2006), and RefFinder (Taki et al., 2014). The geNorm algorithm expresses gene expression stability as M values, the average pairwise variation of each gene compared to that of all other reference genes. Each Cq value is transformed into a deltaCq to form the equation, $2^{(-dCq)}$. Hence, all data are expressed in relation to the expression of the least expressed gene. The optimal number of reference genes for normalization is determined with the V_n/V_{n+1} formula between a combination of sequential normalization factors (NF_n and NF_{n+1}). The NF calculation is based on the geometric means of several reference genes and NFs step wisely add gene numbers until $V_{n/n+1} \leq 0.15$ (Vandesompele et al., 2002). The NormFinder algorithm uses a mathematical model to rank expression stability values from measurement of overall gene expression variation and variation across subgroups of each gene. Each Cq value is log transformed (natural base (e) logarithm) before calculation (Andersen et al., 2004). Bestkeeper ranks expression stability of reference genes according to their average standard deviation (SD) calculated from quantification cycle (Cq) (or threshold cycle, Ct). The BestKeeper Index, a geometric mean of Cq values of all candidate reference genes, is used to estimate correlations between the reference genes and the index with the Pearson correlation coefficient (*r*) and the probability *P* value. BestKeeper can also analyze sample integrity as an intrinsic variance (InVar) value (Pfaffl et al., 2004). In the comparative dCt method, the expression stability of reference genes is ranked according to average SD values of different Cq values of each reference gene when pairwise analysis with other reference genes in all samples tested is performed (Silver et al., 2006). RefFinder is a web-based comprehensive tool. It uses raw Cq values of each sample to search for the most stable gene selection online by comparison of the geNorm, NormFinder, BestKeeper and comparative dCt methods, and makes comprehensive gene stability ranking by calculating weight to each gene and a geometric mean of their weights for all ranking genes is presented (Taki et al., 2014).

Table 3 Ranking of reference genes in all canine oral tumors from high to low stability by various algorithms. Stability values are in brackets.

Rank	geNorm	NormFinder	BestKeeper	dCt	RefFinder
1	RPS5/RPS19 (0.717)	ACTB (0.009)	ACTB (1.05)	RPS519 (1.20)	RPS19 (1.32)
2	-	RPS19 (0.010)	GAPDH (1.18)	ACTB (1.24)	ACTB (1.86)
3	ACTB (1.031)	RPS5 (0.013)	RPS19 (1.19)	RPS5 (1.33)	RPS5 (2.59)
4	RPL13a (1.179)	RPL13a (0.020)	B2M (1.23)	RPL13a (1.48)	RPL13a (4.43)
5	GAPDH (1.316)	GAPDH (0.020)	RPS5 (1.29)	B2M (1.58)	GAPDH (4.56)
6	B2M (1.412)	B2M (0.021)	RPL13a (1.31)	GAPDH (1.60)	B2M (4.73)

Table 4 Ranking of the reference genes in all canine oral tumors from high to low stability with geNorm and NormFinder algorithms, calculated by the web application (http://fulxie.0fees.us/?type=reference&ckattempt=1). Stability values are in brackets.

Rank	geNorm	NormFinder
1	RPS5/RPS19 (0.709)	RPS19 (0.606)
2	-	ACTB (0.634)
3	ACTB (1.025)	RPS5 (0.901)
4	RPL13a (1.169)	RPL13a (1.113)
5	B2M (1.305)	B2M (1.277)
6	GAPDH (1.405)	GAPDH (1.323)

Table 5 Ranking of reference genes in canine OM from high to low stability by various algorithms. Stability values are in brackets.

Rank	geNorm	NormFinder	BestKeeper	dCt	RefFinder
1	RPS5/RPS19 (0.609)	ACTB (0.005)	RPS19 (0.86)	RPS5 (1.02)	RPS5 (1.32)
2	-	RPS5 (0.007)	GAPDH (0.87)	RPS19 (1.04)	RPS19 (1.57)
3	ACTB (0.770)	RPS19 (0.008)	RPS5 (0.90)	ACTB (1.05)	ACTB (3.08)
4	RPL13a (0.895)	RPL13a (0.015)	RPL13a (0.95)	RPL13a (1.20)	GAPDH (3.58)
5	B2M (0.984)	GAPDH (0.016)	ACTB (0.99)	GAPDH (1.33)	RPL13a (4.00)
6	GAPDH (1.189)	B2M (0.024)	B2M (1.22)	B2M (1.59)	B2M (6.00)

The web application (http://fulxie.0fees.us/?type=reference&ckattempt=1) was used to calculate stability values of RefFinder and other algorithms. The stability values of each algorithm calculated from the web application were compared to those calculated by the original software. For all algorithms, the highest stably expressed gene has the lowest stability values (M values, average SD values, etc.). The integrity of each sample was determined by InVar values, using the BestKeeper software. Strong deviating samples due to technical errors such as sample degradation and incomplete reverse transcription were discarded.

Results

Based on the integrity of cDNA samples determined by the BestKeeper algorithm, 1 amelanotic melanoma stage 3, 1 well differentiated OSCC, 1 poorly differentiated OSCC, 1 epulis and 1 hemangiosarcoma were excluded to increase consistency and reliability of the data analysis. For the analysis of suitable reference genes in canine oral tumors, a cohort of top three genes from most algorithms were *RPS5, RPS19* and *ACTB* (Table 3). A similar combination was obtained when these algorithms were calculated by the web application (Table 4). However, the top three reference genes from the geNorm and BestKeeper analyses were a cohort of *RPS5, RPS19* and *RPL13a* (GeNorm), and *ACTB, GAPDH* and *B2M* (BestKeeper) when samples with high InVar values were included.

The geNorm analysis suggested that the top three genes, *RPS5, RPS19* and *ACTB*, had the high expression stability with M values lower than the cut-off value at 1.5 (Vandesompele et al., 2002). $V_{2/3}$-$V_{5/6}$ scores of all OM and/or OSCC ranged from 0.209-0.345. Since the V scores were higher than 0.15, this study suggested a combination of the three best reference genes (*RPS5, RPS19* and *ACTB*) for further normalization to the target gene expression as recommended by the geNorm manual (medgen.ugent.be/~jvdesomp/genorm/geNorm_manual.pdf). From the pair-wise correlation analyses, it was found that all reference gene studied showed strong correlation with the BestKeeper Index, the geometric mean of Cp values of candidate reference genes, ($p \leq 0.001$) with high coefficient of correlation ($0.609 < r < 0.910$).

When the groups of tumors were separately analyzed as OM, OSCC and benign groups (Tables 5-7), a cohort of *RPS5, RPS19* and *ACTB* was still at the highest rank of reference genes except the results from the GeNorm algorithm in OSCC, where RPS5, RPS19 and RPL13a were shown up as from BestKeeper algorithm. The BestKeeper algorithm also showed aberrant ranking results in the OM and benign groups.

Discussion

Since the validity of the qRT-PCR results is also dependent on the reference gene used, the selection of suitable reference genes is highly important. In this study, the suitable reference genes for canine oral tumors were suggested, using various statistical algorithms. Excluding high InVar values and the Bestkeeper algorithm, most algorithms represented the cohort of *RPS5*, *RPS19* and *ACTB* as the top three reference genes for all oral tumor, OM, OSCC and benign groups. *RPS5* and *RPS19* were reported to be the most appropriate reference genes in canine brain (Park et al., 2013), canine skin (Schlotter et al., 2009), canine cell lines and several tissue samples (Brinkhof et al., 2006). *ACTB* was selected to be a stable reference gene in several mouse models (Gong et al., 2014; Yuanyuan et al., 2015; Ma et al., 2014). Interestingly, GAPDH, one of the most commonly used reference genes in several publications (Hernández et al., 2015; Li et al., 2015; Park et al., 2013) did not achieve the highest rank when analyzed by several algorithms. However, GAPDH did appear when using the BestKeeper algorithm. The divergent ranks of the top three genes calculated by different normalization approaches revealed the necessity to use more than one algorithm to reduce bias. This study found that the web application gave similar rank of candidate reference genes as the original algorithms, making this tool feature useful in practice. The RefFinder was also used in selection of reference genes in chicken tissues (Bagés et al., 2015), Nile tilapia (Wang et al., 2015), human cancer cell lines (Jacob et al., 2013; Liu et al., 2015), and mouse testis (Gong et al., 2014). Since the top three reference genes always changed when samples with high InVar values were included, it is important to verify the sample's integrity before the selection of normalization methods.

Conclusion

This study validated the combination of *ACTB*, *RPS5* and *RPS19* as the optimal reference genes for qRT-PCR analysis of canine oral tumor tissues. The combination of reference genes and the utilization of more than one algorithm are recommended. These reference genes will standardize experiments across different laboratories and increase the reliability of any qRT-PCR gene expression analysis of canine oral tumor tissues in clinical diagnosis, prognosis, drug testing, etc.

Table 6 Ranking of reference genes in canine OSCC from high to low stability by various algorithms. Stability values are in brackets.

Rank	geNorm	NormFinder	BestKeeper	dCt	RefFinder
1	RPS5/RPS19 (0.818)	ACTB (0.004)	RPS5 (0.97)	ACTB (1.11)	RPS19 (1.68)
2	-	RPS19 (0.012)	RPS19 (1.04)	RPS19 (1.24)	RPS5 (1.73)
3	ACTB (1.041)	RPS5 (0.013)	RPL13a (1.08)	RPS5 (1.29)	ACTB (2.00)
4	RPL13a (1.126)	GAPDH (0.016)	ACTB (1.17)	RPL13a (1.36)	RPL13a (3.46)
5	GAPDH (1.239)	RPL13a (0.018)	B2M (1.20)	GAPDH (1.37)	GAPDH (5.23)
6	B2M (1.346)	B2M (0.019)	GAPDH (1.38)	B2M (1.51)	B2M (5.73)

Table 7 Ranking of reference genes in canine oral benign tumors from high to low stability by various algorithms. Stability values are in brackets.

Rank	geNorm	NormFinder	BestKeeper	dCt	RefFinder
1	RPS5/RPS19 (0.810)	RPS19 (0.007)	ACTB (0.83)	RPS19 (1.10)	RPS19 (1.32)
2	-	ACTB (0.009)	GAPDH (0.88)	ACTB (1.20)	ACTB (1.86)
3	ACTB (0.939)	RPS5 (0.011)	RPS19 (1.01)	RPS5 (1.26)	RPS5 (2.45)
4	B2M (1.077)	B2M (0.015)	RPS5 (1.09)	B2M (1.35)	B2M (4.23)
5	RPL13a (1.227)	GAPDH (0.020)	B2M (1.24)	RPL13a (1.53)	GAPDH (4.56)
6	GAPDH (1.345)	RPL13a (0.023)	RPL13a (1.38)	GAPDH (1.59)	RPL13a (5.23)

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บทคัดย่อ

การคัดเลือกยีนอ้างอิงสำหรับการทำปฏิกิริยาลูกโซ่พอลิเมอเรสเรียลไทม์ ในเนื้องอกในช่องปากสุนัขชนิดธรรมดาและมะเร็ง

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เนื้องอกในช่องปากทั้งชนิดธรรมดาและมะเร็งพบได้บ่อยในสุนัข แม้จะมีการศึกษาการแสดงออกของยีนระดับเอ็มอาร์เอ็นเอที่เกี่ยวข้องกับโรคในช่องปากมากขึ้น แต่ยังไม่มีการรายงานถึงการคัดเลือกยีนอ้างอิงที่เหมาะสมเพื่อใช้วิเคราะห์การแสดงออกของยีนเป้าหมาย การศึกษาครั้งนี้จึงมีจุดประสงค์เพื่อคัดเลือกยีนอ้างอิงสำหรับโรคเนื้องอกในช่องปากสุนัข อาทิ อีปูลิส อะเมลโลบลาส มะเร็งช่องปากเมลาโนมา มะเร็งช่องปากสแควมัสเซลล์คาร์ซิโนมา ฯลฯ ยีนอ้างอิง 6 ยีนที่ทำการศึกษา ได้แก่ เบตาแอคติน เบตาทูไมโครกลอบูลิน กลีเซอรอลดีไฮด์ทรีฟอสเฟตดีไฮโดรจีเนส ไรโบโซมอลสับยูนิต L13a ไรโบโซมอลโปรตีน S5 และไรโบโซมอลโปรตีน S19 โดยใช้โปรแกรมวิเคราะห์ 5 โปรแกรม ได้แก่ ยีนอม นอมฟายเดอร์ เบสคีบเปอร์ การเปรียบเทียบค่าเดลตาซีที และเรฟฟายเดอร์ ผลปรากฏว่าโปรแกรมส่วนใหญ่ยกเว้นเบสคีบเปอร์แสดงว่ากลุ่มของไรโบโซมอลโปรตีน S5 ไรโบโซมอลโปรตีน S19 และเบตาแอคตินเหมาะสมที่จะใช้นอร์มัลไลซ์ยีนเป้าหมายต่อไป เนื่องจากโปรแกรมวิเคราะห์บางโปรแกรมแสดงลำดับยีนอ้างอิงไม่เหมือนกัน ดังนั้นการใช้ยีนอ้างอิงมากกว่า 1 ยีนและการใช้โปรแกรมประเมินความเหมาะสมของยีนอ้างอิงมากกว่า 1 โปรแกรมจึงมีความสำคัญ สรุปได้ว่าจากการศึกษาครั้งนี้การใช้เบตาแอคตินร่วมกับไรโบโซมอลโปรตีน S5 และไรโบโซมอลโปรตีน S19 มีความเหมาะสมที่จะใช้เป็นยีนอ้างอิงในการศึกษาการแสดงออกของยีนในเนื้องอกในช่องปากสุนัขต่อไป ยีนอ้างอิงดังกล่าวจะเพิ่มความน่าเชื่อถือในการวิเคราะห์การแสดงออกของยีนด้วยปฏิกิริยาลูกโซ่พอลิเมอเรสเรียลไทม์แบบย้อนกลับในโรค เนื้องอกและมะเร็งในช่องปากสุนัขในด้านต่างๆ อาทิ การวินิจฉัยโรคบนคลินิก การพยากรณ์โรค และการทดสอบยา

คำสำคัญ: สุนัข มะเร็งในช่องปาก เนื้องอกในช่องปาก ปฏิกิริยาลูกโซ่พอลิเมอเรสเรียลไทม์แบบย้อนกลับ ยีนอ้างอิง

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