Involucrin Expression and Association with Ki-67 in Paraffin Embedded Tissue of Canine Skin Tumors: A Retrospective Study

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Abstract

Involucrin (INV) is a cornified envelope precursor protein that is commonly used as an early stage marker of human terminal keratinocyte differentiation. This research investigated the expression of INV in canine skin tumors using 56 formalin-fixed paraffin-embedding (FFPE) biopsied specimens. The expression of INV was investigated by immunohistochemistry (IHC) and RT-PCR. Correlation between INV and Ki-67 was also performed. The malignant tumor groups showed significantly different INV staining scores and Ki-67 positive cells than the normal skin group by IHC ($p<0.05$). The INV scores of moderately differentiated SCC (MSCC), trichoblastoma (TCB), basal cell carcinoma (BCC) and poorly differentiated SCC (PSCC) significantly decreased, with a statistically significant difference ($p<0.05$). Interestingly, the INV of poorly differentiated SCC disappeared in some specimens. The INV expression in most tumors was found to be inversely proportionate to Ki-67 positive cells. In addition, only SCC regardless of tumor grade without tumor-graded specimens showed an inversely statistically significant difference between the Ki-67 positive cells and INV expression ($p<0.0001$). In RT-PCR, mRNA expression of the FFPE specimens showed substantial similarity of INV expression as in IHC. It is suggested that the INV expression should be further studied in terms of clinical prognosis in canine cutaneous SCC.

Introduction

Thirty-five years ago, Involucrin (INV, Latin for envelope: *involucrum*) was known as the major small-soluble protein of a cornified envelope (Rice and Green, 1977; Eckert et al., 1997). Because INV is specifically expressed in keratinocyte undergoing terminal differentiation in the supra-basal layer of skin, it has been commonly used as a marker for terminally differentiated human keratinocytes at the early stage (Eckert et al., 1997; Li et al., 2000; Balasubramanian and Eckert, 2004). Generally, the structure of INV in mammalian placenta is conserved across animal species. Its structure consists of a homologous segment of 10 amino acids in repeating sequences. Each repeating unit contains 3 glutamine residues; however, the number of repeating segments varies according to species (Tseng and Green, 1990).

Since INV greatly contains large amounts of glutamine residues on its molecular structure, these amino acid residues are catalyzed by transglutaminase to create insolubly cross-linked envelopes beneath the inner membrane surface of keratinocytes (Eckert et al., 2004). These cross-links also play a critical role as the scaffold for stratified squamous epithelium to which a family of waxy lipids and ceramides is attached (Li et al., 2000; Nishifuji and Yoon, 2013). In 1990, Tseng and Green explained that human beings, dogs and pigs similarly possessed INV in the terminally differentiating keratinocytes; however, they were significantly different from one another in the codon sequence, depending on species. Therefore, the structure of INV is particularly important for the reason that it may determine the functions of INV.

INV has largely been reported and used as a marker in both dermatological and oncological research studies. It has been used not only as an early stage marker of human terminal keratinocyte differentiation, but also as an indicator for wound re-epithelialization enhancement (Obedencio et al., 1999), a marker for atopic lesion with a reduction in non-apoptotic cell proliferation (Hertle et al., 1992; Jensen et al., 2004), a premalignant lesion marker (Caldwell et al., 1997; Li et al., 2000; Balasubramanian and Eckert, 2004; Nozoe et al., 2006) and an endogenous marker for hypoxia of squamous cell carcinoma (Chou et al., 2004) as well. As in humans, the incidence of canine neoplasms worldwide has reportedly increased and the skin has become one of the most prevalent sites for neoplasm development (Dobson, 2011). Even though various markers of human cancers have been applied in veterinary oncology, some including INV are still questionable with limited information about their use in veterinary practice.

Kozaki et al. (2001) reported that human INV-l monoclonal antibodies were not appropriate in dog skin and showed negative immunoreactions in INV immunohistochemistry of epidermis, hair follicles, sweat gland ducts and canine epithelial neoplasm. Interestingly, in one INV-IHC study of normal dog skins using mouse INV monoclonal antibodies (Clone SY5, Abcam, UK), the result showed that the staining scores of skin specimens did not show any specificities to the anatomic sites, breed and gender (Theerawatanasirikul et al., 2012a). In addition, the INV-mRNA expression from biopsied skin specimens demonstrated that it could be a powerful tool for canine atopic dermatitis diagnosis (Theerawatanasirikul et al., 2012b). However, the utilization of INV as a diagnostic and prognostic marker for canine skin cancers is still vague, although it could be conceivable that canine INV expression correlates with development levels in canine neoplasms as in humans, especially canine SCC.

MKI67 (Ki-67), a proto-oncogene which regulates cell proliferation, is usually utilized as a cell proliferation index marker because of its typically increasing presence in most malignant neoplasms (Scholzen and Gerdes, 2000) and its relation to rapid growth and recurrence in non-melanotic skin cancer (NMSC) (Noezoe et al., 2006; Martins, et al., 2009) and poor prognosis in canine SCCs (Sailasuta et al., 2012).

RNA analysis is an important tool to study gene expressions in almost all cancer research since it can be used as a prognostic, diagnostic and therapeutic marker for all cancers (Penland et al., 2007). Although the use of FFPE specimens for RNA evaluation is still questionable because in FFPE samples RNA usually undergoes chemical modification by formalin and degrades over time, FFPE specimens are still beneficial to retrospective studies (Benchekroun et al., 2004).

The aim of this study was to investigate the expression level of INV protein in formalin-fixed paraffin-embedding (FFPE) specimens of canine cutaneous SCC and other skin neoplasms. The expression levels of INV protein were investigated by INV-IHC and RT-PCR. Moreover, correlation between INV protein expression and Ki-67, a proliferative marker of cancer (Scholzen and Gerdes, 2000), was performed. Additionally, clinical information was discussed.

Materials and Methods

Tissue samples: Data were provided through a retrospective study of tissue sample of skin neoplasm biopsied from dog patients diagnosed from 2009 to 2010 by pathologists at the Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University. The FFPE specimens obtained by biopsy were selected and information on clinical data was retrieved from the department's database. In the study of INV and Ki-67 expressions, 4 normal skin samples at the ventral aspect of the absent skin of diseased dogs were used as the normal control and the samples were obtained from the department's necropsy room.

Histology and immunohistochemistry of INV and Ki-67: All FFPE biopsied tissues had been re-cut and routinely processed for hematoxylin-eosin (H&E) for light microscopy. Histopathological examination was undertaken according to a classification system recommended by WHO (Goldschmidt MH and Hendrick, 2002).

A serial section of each specimen was prepared for Ki-67 and INV-immunohistochemistry using standard protocols previously described (Theerawatanasirikul et al., 2012b). Briefly, each deparaffinized tissue section was incubated with citrate buffer (0.01 M, pH 6.0) at 95°C for 40 min for Ki-

67 antigen retrieval and trypsinized by 1.0% trypsin for 15 min for INV antigens. The serial samples were incubated with Mouse-monoclonal anti-human involucrin (Clone SY5, Abcam, UK) at dilution of 1:1000 and anti-human Ki-67 antibodies (Clone MIB-1, DAKO, Denmark) at dilution of 1:200, then incubated by a polymer-based non-avidin-biotin system (EnVision™, Dako, Denmark) and colorized using 3, 3′-diaminobenzidine tetrahydrochloride (DAB) as the chromogenic substrate (Zymed Laboratories, UK). Positive and negative control slides were prepared using canine skin with and without primary antibodies, respectively. Intensity of INV-immunopositivity was performed on 10 randomly selected fileds and analyzed using an image analysis progress (Image-Pro PLUS 6.0 programming) (Media Cybernetics, USA). The cytoplasmic staining intensity was semi-quantitatively scored as 0 (negative), 1 (weak), 2 (moderate), and 3 (strong). Percentage of positive area was evaluated from division of positive cytoplasmic staining (mm^2) per total area of epidermis (mm^2). The ratio of positive area in combination with intensity score was calculated as 'staining score'. The staining scores were calculated from the summation of (intensity score x percentage of area stained in each level) divided by 100 and mean of the total scores was calculated and used for analysis. Epidermal proliferation in each tissue section was shown as density of positive cells by counting the number of keratinocytes that were positive for Ki-67 immunoreactivity in nuclei of epidermis. The average number of positive cells was calculated for the epidermis in the unit 'positive cell per linear mm of total epidermal surface length' by using Image-Pro PLUS 6.0 program (Media Cybermatic, U.S.A.) for image analysis (Theerawatanasirikul et al., 2012a).

RNA extraction and reverse transcription-polymerase chain reaction (RT-PCR): Four FFPE biopsied specimens of each neoplasm type were randomized and selected for RT-PCR study. A 25 mg-trimmed tissue from each case was utilized for total RNA extraction using a commercial FFPE-RNA purification kit (Norgen®, Biotek corp., Canada), according to the manufacturer's instructions. Each total RNA solution was incubated by TURBO DNase-free (Ambion®, USA) to diminish genomic DNA. Nanodrop spectrophotometry was performed using NanoDrop™ ND-1000 Spectrophotometer V3.7 (Thermo Fisher Scientific, USA) to determine the concentration and purity in each RNA sample.

A 300 ng solution of total RNA from each specimen was converted to first-strand cDNA which was amplified using a one-step RT-PCR kit (SuperScript™ III, Invitrogen, USA) with Taq-polymerase (Platinum®-Taq, Invitrogen, USA). The pair of primers for canine INV-mRNA used in this study followed that of Theerawatanasirikul et al. (2012a) which consisted of 5′AAA GAA GAG CAG GTG CTG GA 3′ (forward) and 5′TGC TCA CTG GTG TTC TGG AG 3 (reverse). This pair of primers was setup from the referral mRNA sequence reported on http://frodo.wi.mit.edu/ using computational software Primer 3™ version 0.4.0. The predicted PCR product size was 203-bp. Canine GAPDH was used as a housekeeping gene and was amplified using the set of primers as follows: forward-5' AGT CAC TGC CAC CCA GAA GAC 3' and reverse-5' GGC AGG TCA GAT CCA CAA CGT 3' with the prediction of 202-bp in its production size.

The tumor samples were used for RT-PCR and run in a 96-well GeneAmp™thermocycler 9700 (Applied Biosystems, USA) at different temperatures: 53ₒC for 30 sec (cDNA synthesis), 94ₒC for 2 min (initial denaturation), 35 cycles of 94ₒC for 15 sec, 53ₒC for 30 sec and 68ₒC for 30 sec, with a final extension at 68ₒC for 5 min. Ten µl of each RT-PCR product were size-fractionated by 1% agarose gel electrophoresis in 1X TAE buffer at 110 Volts for 25 min and stained by 1% ethidium bromide. Finally, the products were visualized using a Gel Doc™ XR⁺ documentary machine (BioRad, USA). Band intensity of INV mRNA was measured by accessory software that had been installed for the Gel Doc™.

Statistical analysis: The INV and Ki-67 IHC staining parameters were exhibited in terms of mean and standard deviation (SD), depending on the neoplastic types. A statistical analysis of immunohistochemical staining and correlation of determination (R^2) were performed using a GraphPad Prism software, version 5.0 (San Diego, CA, USA). One-way ANOVA and post-hoc test as well as parametric value by the Tukey-Kramer test were performed to determine differences among skin neoplasm types analyzed in this study. Dunn's significant differences were determined by Dunn's Multiple Comparison Test. Additionally, simple linear-regression analysis was employed in selected groups that showed preliminary correlation between INV and Ki67.

Results

Clinical profiles of skin neoplasm in dog patients: The examination of 56 FFPE biopsied skin neoplasm of dog patients was undertaken using the classification system recommended by the WHO (Goldschmidt and Hendrick, 2002), consisting of 25 benign skin tumor samples; trichoblastoma (10), papilloma (10), basosquamous cell carcinoma (5), and 31 malignant skin tumor samples; basal cell carcinoma (n=10), well-differentiated SCC (8), moderately differentiated SCC (6) and poorly differentiated SCC (7). Almost all patients were mixed breed (51.8%) and the second most represented breed was poodle (19.6%). The average age was 9.4 years. Skin neoplasms were found in dogs over 7 years old, but papilloma was seen in 5-year-old dogs.

Immunohistochemical staining pattern of INV and Ki-67 in FFPE biopsied specimens and their relationship in canine skin neoplasms: Based on INV-immunohistochemistry in normal epidermis, INV-immunoreactivity was obviously positive in the cytoplasm of the keratinocytes at the stratum basale. However, its intensity was liable to reduce in those cells within the upper most layers. Moreover, the staining score in image analysis demonstrated a significant difference in this parameter among the studied neoplasms. The INV staining scores of

malignant skin tumors (basal cell carcinoma and all graded SCC), benign skin tumors (papilloma, basosquamous cell carcinoma and trichoblastoma) and normal skin were 0.49+0.096, 0.77+0.09, and 0.81+0.10, respectively. It was demonstrated that the INV staining scores of the malignant skin tumors were the lowest compared to the benign skin tumors and the normal skin with a statistically significant difference ($p<0.05$) (Fig 1a). For the skin tumor types, moderately differentiated SCC was 0.73+0.11, poorly differentiated

SCC was 0.077+0.09, basal cell carcinoma was 0.35+0.07 and trichoblastoma was 0.55+0.07, which showed significant decrease in INV staining scores when compared to the normal skins (0.92+0.10) ($p<0.05$). Interestingly, the staining scores of poorly differentiated SCC disappeared in some specimens and were statistically different compared to the other tumors ($p<0.05$) (Fig 1b). Additionally, the INV staining score might be associated with the grade of SCC and types of skin tumor.
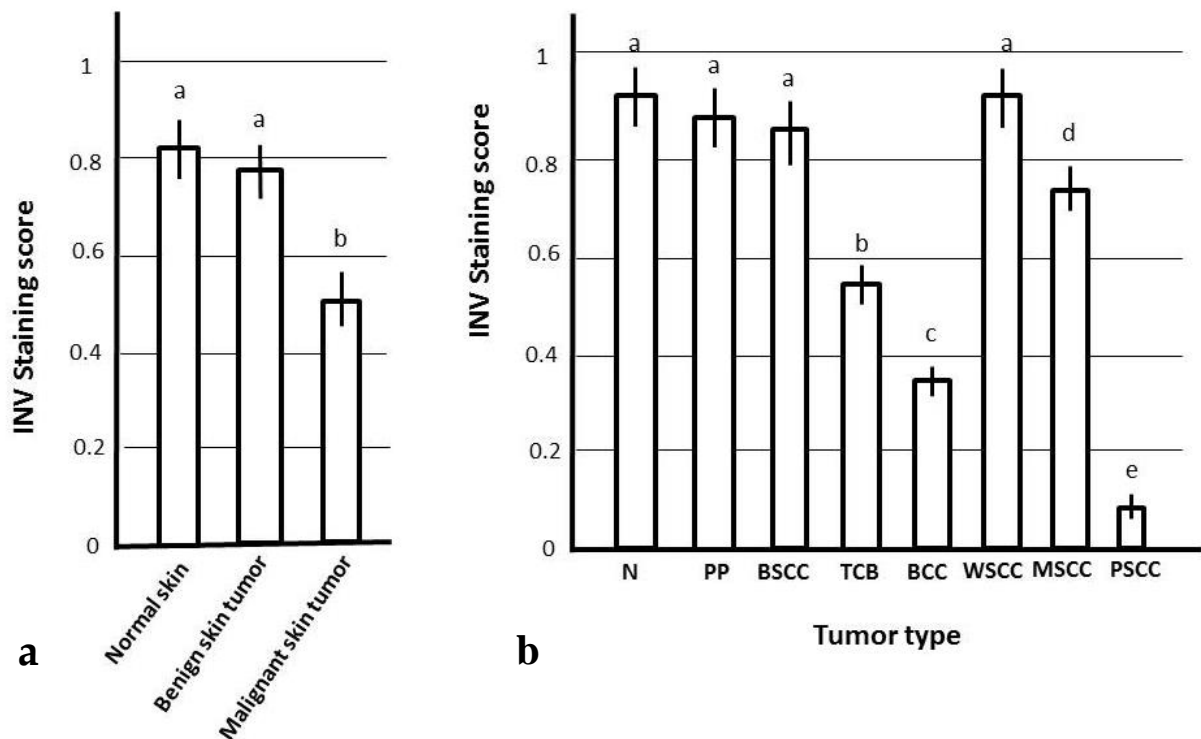

Figure 1 **INV staining score** (a) INV staining scores in normal skin, benign skin tumors and malignant skin tumors. (b) INV staining score in normal and various canine skin neoplasm types. The INV expression in normal skin (N), well-differentiated SCC (WSCC), basosquamous cell carcinoma (BSCC) and papilloma (PP) groups was significantly stronger than in the other groups ($p<0.05$). The INV score of moderately differentiated SCC (MSCC), trichoblastoma (TCB), basal cell carcinoma (BCC) and poorly differentiated SCC (PSCC) significantly decreased, with a statistically significant difference ($p<0.05$). Different alphabets (a, b, c, d, e) show significant statistical difference ($p<0.05$).

The Ki-67 positive cells (shown as brown dots) were present in the nuclei of the cancerous cells. The results showed that the number of positive cells in the malignant skin tumor (basal cell carcinoma and all graded SCC) was 86.12+58.51, benign skin tumor (papilloma, basosquamous cell carcinoma, trichoblastoma) was 8.96+7.33 and normal skin was 10.04+4.78. It was demonstrated that the Ki-67 positive cells of the malignant skin tumors were the highest compared to the benign skin tumors and the normal skin with a statistically significant difference ($p<0.05$) (Fig 2a). For the skin tumor types, the expression was most obvious in the poorly differentiated SCC (218.8+105.0), followed by the trichoblastoma (14.43+10.52), basal cell carcinoma (77.00+37.86), moderately differentiated SCC (15.00+15.97), well-differentiated SCC (33.50+26.05), papilloma (7.50+6.09), basosquamous cell carcinoma (5.43+6.37) and normal skins (4.64+4.55), respectively (Fig 2b).

Both INV staining score and Ki-67 positive cells correlated with the differentiated and proliferative stages of keratinocytes in neoplastic tissues. INV was strongly expressed in the well-differentiated SCC cells. The increase in Ki-67 positive cells was found in the poorly differentiated SCC. In other words, INV expression was inversely proportionate to Ki-67 positive cells, except in the basal cell carcinoma. According to the statistical analysis with Spearman rank correlation test, the result indicated that no statistical significance among the groups was shown. However, when focusing on the SCC group without the tumor-graded specimens, the correlation test showed a significant inverse relation between the Ki-67 positive cells and INV staining score ($p<0.0001$, $R^2=0.70$). The dot-plot of linear-regression was used for the confirmation of INV and Ki67 among the SCC groups as shown in Figure 3.
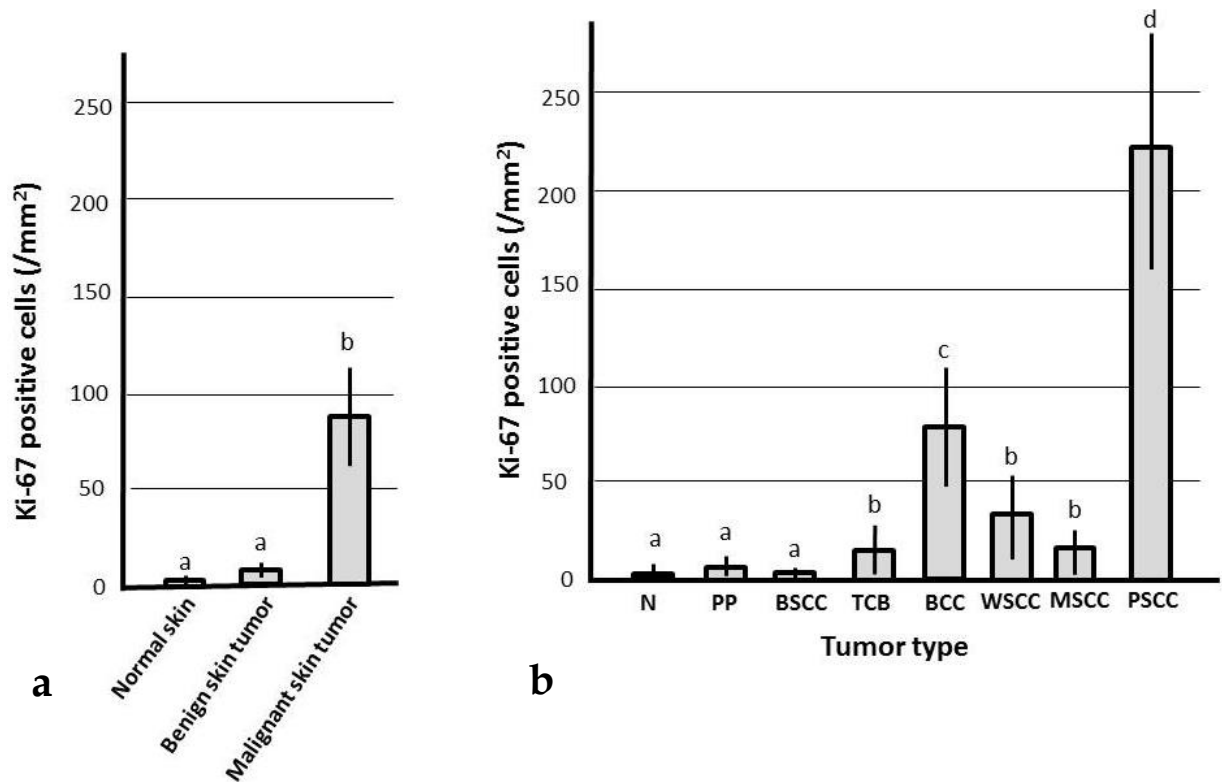
Figure 2 **Density of Ki-67 positive cells** (a) Ki-67 positive cells in normal skin, benign skin tumors and malignant skin tumors. (b) Ki-67 positive cells in normal and various types of canine skin tumor. The Ki-67 positive cells in normal skin (N), well-differentiated SCC (WSCC), papilloma (PP) and basosquamous cell carcinoma (BSCC) groups were significantly lower than in the other groups ($p<0.05$). The trichoblastoma (TCB), well-differentiated SCC (WSCC), and moderately differentiated SCC (MSCC) significantly increased. Basal cell carcinoma (BCC) was higher than the others, and poorly differentiated SCC (PSCC) was the highest ($p<0.05$). Different alphabets (a, b, c, d) show significant statistical difference ($p<0.05$).
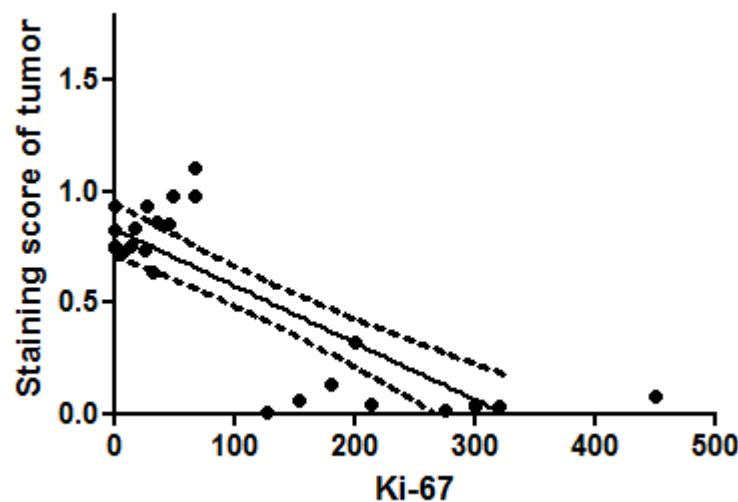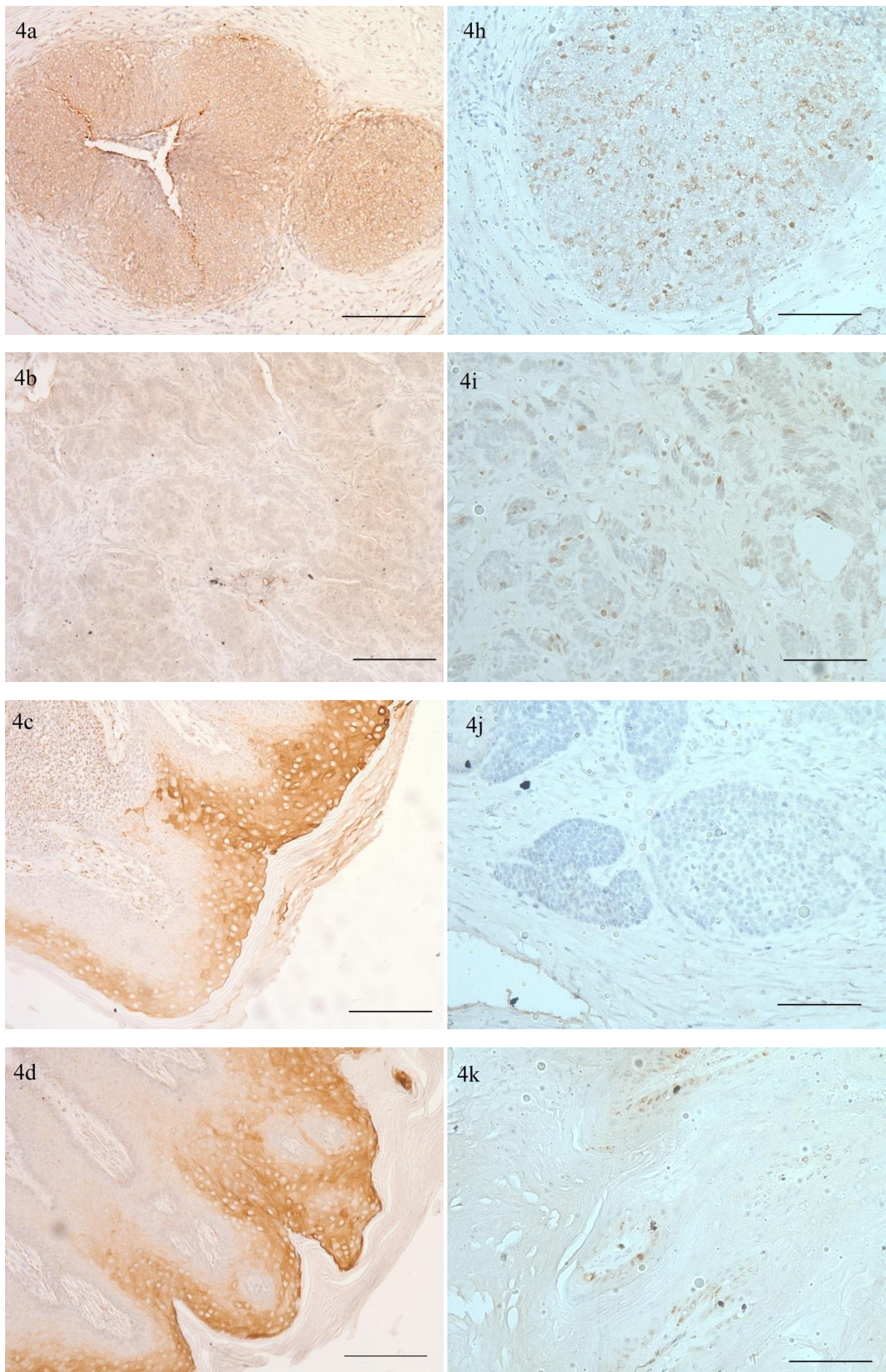

Figure 3 **Relationship between Ki-67 and INV staining score in SCC without tumor-graded specimens.** Plots of linear-regression analysis are shown. The dotted lines depict 95% confidence bands for means ($R^2=0.70$).

Involucrin-mRNA expression in skin tumor: As expected, 200-bp amplicons of INV were observed in all specimens with varied intensity. The expression was remarkably dominant in the normal skin, in which the band intensity was approximately 3.47 folds when compared to the ladder. Moreover, the band intensity seemed to correlate with neoplastic types and its degree of cellular differentiation. For instance, the well-differentiated SCC had 2.12 folds of band intensity, while the papilloma, basosquamous cell carcinoma, trichoblastoma, moderately differentiated SCC, and basal cell carcinoma had 2.05, 1.95, 1.74, 0.94, and 0.84 folds, respectively. The lowest intensity was observed in the poorly differentiated SCC (0.79), as shown in Figure 5.
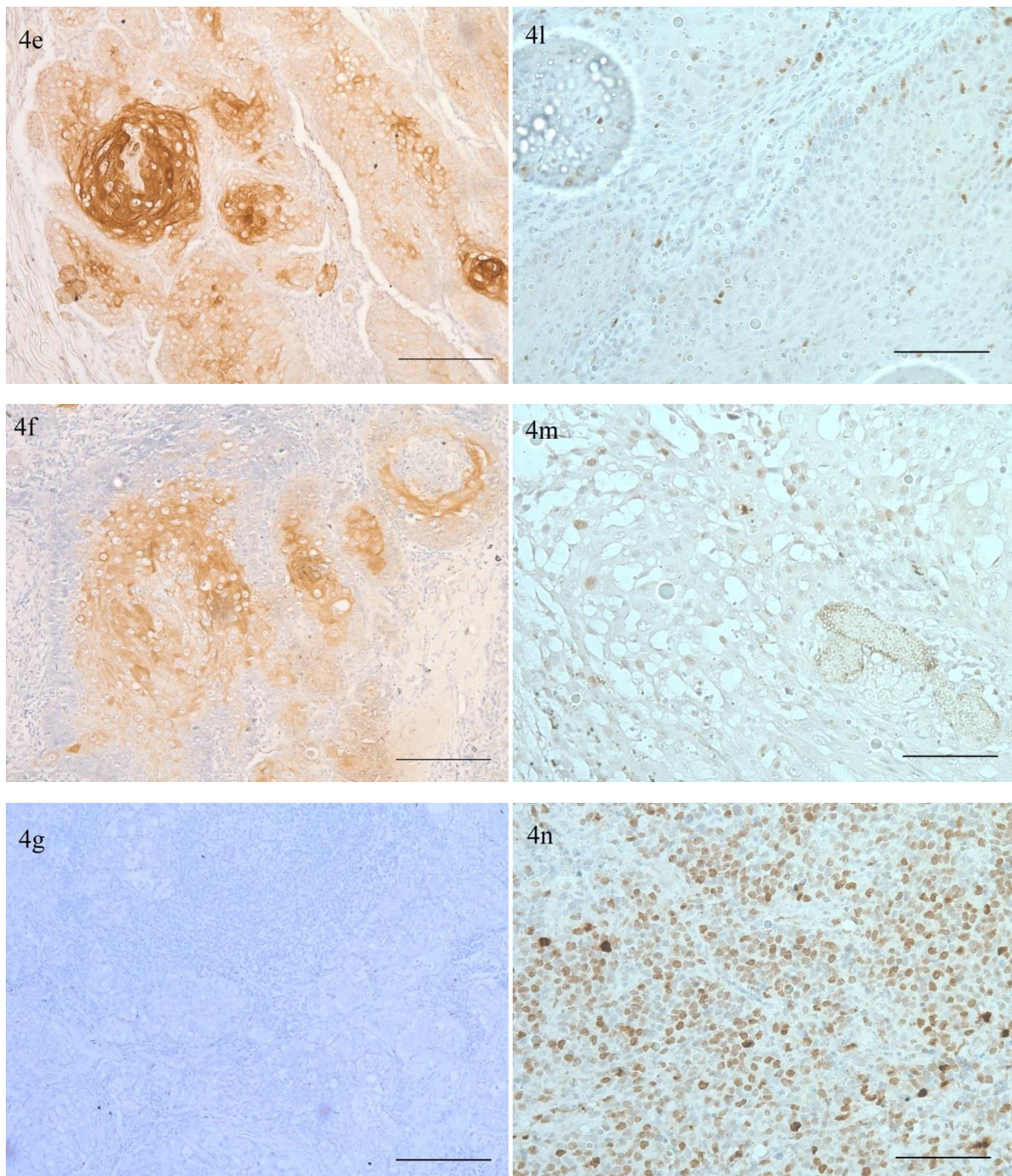
Figure 4   Immunohistochemical staining of Involucrin (left column) and Ki-67 positive cells (right column). Basal cell carcinoma (4a, 4h), trichoblastoma (4b, 4i), basosquamous cell carcinoma (4c, 4j), papilloma (4d, 4k), well-differentiated SCC (4e, 4l), moderately differentiated SCC (4f, 4m), poorly differentiated SCC (4g, 4n), IHC, Envision™, Mayer's Hematoxylin counterstained, DAB, Bar=200 μm

Discussion

IHC of various proteins has been used widely for neoplastic diagnosis and prognosis, and almost all studies have been performed on FFPE specimens (Webster et al., 2009). This study demonstrates the success in using various FFPE biopsied neoplastic samples to evaluate INV expression by IHC and RT-PCR of the mRNA level. The result suggests that FFPE biopsied samples are useful for long-term retrospective studies, thus the limitation of frozen samples is overcome (Körbler et al.,

2003). Basically, INV is expressed in the terminally differentiated epithelial cells, but poorly expressed in undifferentiated cells. Therefore, INV expression is a possible diagnostic and prognostic marker for premalignant lesions of keratinocytes and SCC in humans as well (Murphy et al., 1984; Kanitakis et al., 1986; Watanabe et al., 1995; Caldwell et al., 1997; Li et al., 2000; Lan et al., 2014), and it has long been utilized as a premalignant marker for keratinocytes of human SCC as its expression level seems to significantly correlate with clinico-pathologic signs of SCC patients (Nozoe et al., 2006). Moreover, INV varied on

expression in hypoxic tissues (Chou et al., 2004). Therefore, it has been used as a differentiation marker and an endogenous hypoxic marker for human SCC (Chou et al., 2004). The INV expression in all skin neoplasms in this study demonstrated that INV was significantly decreased, except in the well-differentiated SCC, basosquamous cell carcinoma and papilloma cases. Interestingly, among the SCC groups, the INV expression of the poorly differentiated SCC was clearly statistically decreased. There were are

some previous reports agree with our results and reported in cultured cell (Chou et al., 2004; Martins et al., 2009; Lan et al., 2014). This evidence could possibly be explained via the Activator protein-1(AP-1) transcription factor mechanism, which is an important regulatory protein in epidermis and cell biological processes, such as keratinocyte differentiation, proliferation, apoptosis and transformation in cancer progression, in which one of the differentiation marker is involucrin (Eckert et al., 2004; Rorke et al., 2010).
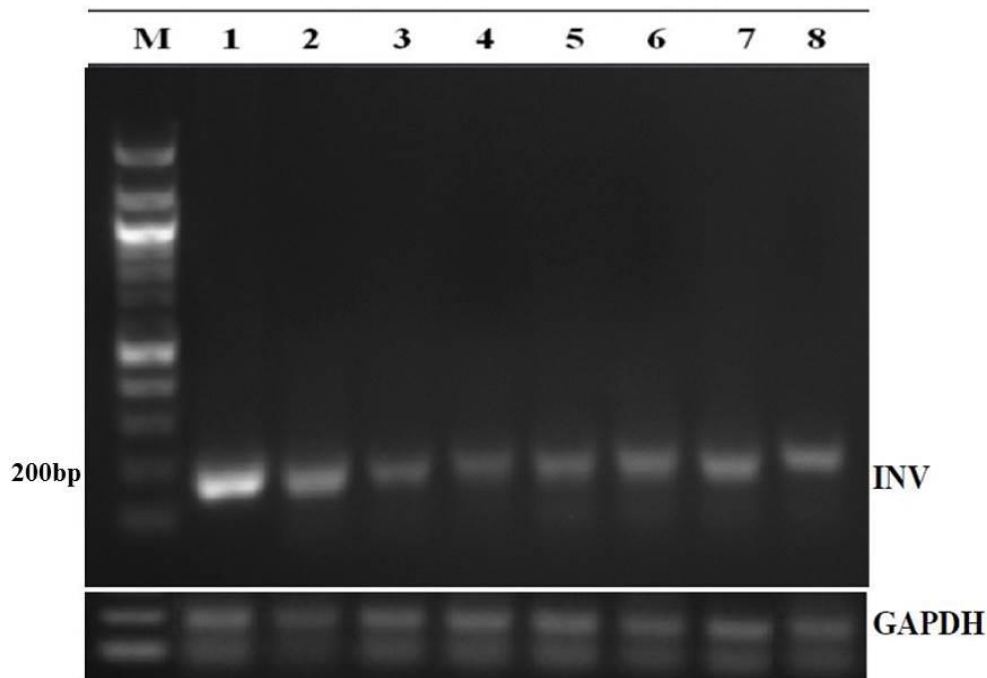

Figure 5   **Agarose gel electrophoresis.** Amplicons (200 bp) of INV-mRNA expression in canine normal skin (Lane 1) was 3.47, well-differentiated SCC was 2.12 (Lane 2), moderately differentiated SCC was 0.94 (Lane 3), poorly differentiated SCC was 0.79 (Lane 4), basal cell carcinoma was 0.84 (Lane 5), trichoblastoma was 1.74 (Lane 6), basosquamous cell carcinoma was 1.95 (Lane 7) and papilloma was 2.05 (Lane 8) folds of intensity of Lane M. Lane M: 100 bp DNA ladder.

The increased expression of Ki-67 is related to rapid growth and recurrence in non-melanotic skin cancer (NMSC) and suggests poor prognosis in malignant skin neoplasms (Nozoe et al., 2006; Martins et al., 2009). According to our results, the Ki-67 positive cells in most skin neoplasms showed reversely INV-IHC expression, especially in the malignancy cases. There was a report on the use of Ki67 and INV ratio as P/D ratio (proliferation to differentiation) to indicate a pre-cancerous state of human skin neoplasm, for example, psoriasis, Bowen's disease, solar keratosis, and verrucous carcinoma and SCC, which were significantly difference (Caldwell et al., 1997). In our results, the reverse correlation between Ki-67 and INV was statistically significant (*p<0.0001*) particularly in the SCC group without tumor-graded specimens by using the Spearman rank correlation. It is suggested that the tumor grade by histopathology may related to their bio-behavior. This study proposed that the number of specimens should be added for further statistical analysis. This propensity increases in cancers with clinically aggressive biological behaviors are similar to the case of human NMSC reported by Nozoe

et al. (2006). Hence, this result suggests the relevance of INV expression in SCC cases and also indicates that INV expression might be a useful marker for evaluating SCC malignancy.

There is number of scientific evidence showing the utilization of FFPE specimens to quantitate RNA expression, particularly when a retrospective study with fresh specimens could not be performed (Specht et al., 2001; Abrahamsen et al., 2003, Penland et al., 2007). Unfortunately, all reports suggested that most PCR methods that used RT-PCR could not quantitate an amplicon when its size was larger than 200 bp with a high quality result (Lewis et al., 2001). In addition, the results from RT-PCR have substantially shown a similarity to INV expression as in IHC. To achieve this, the extraction method seems to be the key factor in the quantification of RNA levels from FFPE biopsied samples. While there have been various methods reported on the purification of RNA from FFPE specimens (Körbler et al., 2003; Penland et al., 2007), this study employed the combination of 3 commercial extraction kits and provided good results on the RNA extraction and purification from FFPE

biopsied samples. The mRNA levels of INV apparently reduced in all poorly differentiated skin cancers, especially canine SCC. The underlying causes for the reduction in INV expression are not completely understood. However, some probable mechanisms have been proposed (Bikle et al., 2002; Tran and Crowe, 2004; Chou et al., 2004; Eckert et al., 2004; Sinitsyna et al., 2010), including the down regulation of INV gene in transformed cells at the post-transcription level, the dedifferentiation of cancerous cell, the total inhibition of the differentiation process and the incorporation of INV gene to intracellular signaling cascades and transcription factors that regulate differentiation-dependent gene expression such as AP-1 (Eckert et al., 1997; Takahashi et al., 1998; Eckert et al., 2004; Tran and Crowe, 2004).

In conclusion, based on the study's results, it is suggested that INV has a trend to be down-regulated in the malignant skin tumors especially in SCC. Furthermore, the results of INV expression and Ki-67 positive cells in ungraded SCC demonstrated obvious relationship which could possibly imply the malignancy status of SCC. However, to elucidate its role in SCC tumorogenesis, it is suggested that INV expression should be comprehensively studied using various methods such as quantification of mRNA and western blot along with assessment of the signalment and survival rate of patients, particularly when INV is used as a prognostic marker for canine SCC. This study does provide basic information that improves the understanding of possible roles of INV usage in canine skin tumor.

Acknowledgements

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บทคัดย่อ

การแสดงออกของอินโวลูครินและความสัมพันธ์ต่อ Ki-67 ในเนื้องอกผิวหนังสุนัขที่ฝังในก้อนพาราฟิน: การศึกษาแบบย้อนหลัง

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อินโวลูคริน (Involucrin, INV) คือโปรตีน cornified envelope precursor ที่ใช้ทั่วไปในการบ่งชี้เคราตินเซลล์ที่มีการเปลี่ยนแปลงในระยะเริ่มต้น งานวิจัยนี้ศึกษาการแสดงออกของ INV จากตัวอย่างชิ้นเนื้อที่ตรึงสภาพด้วยฟอร์มาลินและผ่านกระบวนฝังในพาราฟินของเนื้องอกผิวหนังสุนัขป่วยจำนวน 56 ตัว โดยใช้การย้อมอิมมูโนฮีสโตเคมีและเทคนิค RT-PCR เพื่อศึกษาการแสดงออกของอินโวลูคริน รวมถึงศึกษาเปรียบเทียบการแสดงออกของ INV ร่วมกับเคไอ-67 (Ki-67) การศึกษาพบว่ากลุ่มของเนื้องอกชนิดรุนแรงมีการแสดงออกของ INV และ Ki-67 แตกต่างจากผิวหนังปกติอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) การแสดงออกของ INV ในเนื้องอกชนิด Moderately differentiated squamous cell carcinoma (MSCC), trichoblastoma (TCB), basal cell carcinoma (BCC) **และ** poorly differentiated SCC (PSCC) มีค่าต่ำลงอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) และ PSCC บางตัวอย่างไม่มีการแสดงออกของ INV โดย INV มีแนวโน้มความสัมพันธ์ในการแสดงออกอย่างผกผันกับ Ki-67 เป็นส่วนใหญ่ และมีความสัมพันธ์ผกผันอย่างมีนัยสำคัญทางสถิติในกลุ่มเนื้องอกชนิด SCC ที่ไม่มีการแบ่งระดับความรุนแรง ($p<0.0001$) การแสดงออกของ mRNA ด้วยเทคนิค RT-PCR จากชิ้นเนื้อที่ฝังในก้อนพาราฟินมีผลไปในทิศทางเดียวกับผลการย้อมอิมมูโนฮีสโตเคมี ดังนั้นควรมีการศึกษาความเป็นไปได้ในการใช้การแสดงออกของ INV เพื่อใช้ในการพยากรณ์โรคทางคลินิกของมะเร็งผิวหนังชนิด SCC ของสุนัขในอนาคตต่อไป

คำสำคัญ: สุนัข อิมมูโนฮีสโตเคมี อินโวลูคริน mRNA เนื้อเยื่อฝังในก้อนพาราฟิน เนื้องอกผิวหนัง transcription gene

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