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Comparison of Hedgehog Signaling Proteins in the Serum of Dogs with Mammary Gland Tumors

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Abstract

The hedgehog signaling pathway has been implicated in tumorigenesis and progression of many tumor types. This pathway has recently emerged as a therapeutic target, and inhibitors of hedgehog signaling have gained considerable attention. In dogs, the roles of hedgehog signals in several types of tumors have been investigated, but their relationship with canine mammary gland tumors (MGTs) has not been established. This study aimed to evaluate the expression of sonic hedgehog (SHH) and glioma-associated oncogene 1 (GLI-1) in the serum and mammary tumor tissues of dogs.

SHH and GLI-1 protein expression levels were significantly higher in MGT tissues than in normal mammary gland tissues, as

well as in malignant MGT specimens than in benign MGT specimens. Serum concentrations of SHH and GLI-1 were higher in MGT patients than in healthy controls ($P < .001$ and .001, respectively). Serum SHH level showed a statistically significant relationship with metastatic status ($P = .01$), and serum GLI-1 level showed a statistically significant relationship with histologic grade ($P = 0.048$) and metastatic status ($P = 0.007$). Serum hedgehog signaling protein levels were not significantly associated with breed size, sex, tumor size, or histologic type.

Hedgehog signaling protein expression in canine MGT tissue and serum differed according to the histological classification (benign and malignant) and metastatic status, indicating a relationship between the hedgehog signaling pathway and canine MGT. Thus, the hedgehog signaling pathway may serve as a new biomarker and therapeutic target in canine MGT patients.

Keyword: Hedgehog signal, dog, serum, mammary gland tumor

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Introduction

Mammary gland tumors (MGTs) are the most common neoplasms in intact female dogs, with an incidence of up to 70%. Among patients with MGTs, the incidence of malignant tumors is 53.3%. Prognostic assessment is based on histologic grade, tumor diameter, and lymphatic or vascular invasion of distant metastases (Sorenmo 2003, Vail, Thamm et al. 2019). The treatment of choice for MGTs is surgical resection, except in patients with inflammatory carcinoma or distant metastasis. Systemic treatment can be attempted in high-risk cases, but its efficacy has not been well established (Vail, Thamm et al. 2019).

Hedgehog (Hh) signals play an important role in stem cell differentiation (Bhardwaj, Murdoch et al. 2001, Liu, Dontu et al. 2006), embryonic development (McMahon, Ingham et al. 2003) and tissue regeneration (McMahon, Ingham et al. 2003, Petrova and Joyner 2014). The Hh signaling pathway is activated by canonical and non-canonical mechanisms. The canonical mechanism is dependent on the primary cilium, where membrane receptor proteins and signaling components are concentrated. This pathway begins when the sonic hedgehog (SHH) ligand binds to the membrane receptor patched1 (PTCH1). In the absence of SHH, PTCH1 maintains its interaction with SMO (smoothened), which is inhibited by blocking its displacement to the cell membrane. Upon ligand binding, SMO enters the cell membrane and promotes the transcriptional activity of glioma-associated oncogene 1 (GLI-1). GLI-1 protein activation stimulates the expression of Hh target gene products such as PTCH1, GLI-1, Hh-interacting protein, and cell type-dependent genes such as cyclin D and E, Myx, and Sox2 (P Visbal and T Lewis 2010, Benvenuto, Masuelli et al. 2016, Armas-López, Zúñiga et al. 2017, Galdo, Natalia et al. 2019). In non-canonical Hh signaling, GLI-1 is activated by SMO-independent stimulation (Lauth and Toftgård 2007, Pietrobono, Gagliardi et al. 2019).

The hedgehog pathway is required for normal development of the mammary glands, and is especially essential for the formation of the mammary buds (Hatsell and Frost 2007). The hedgehog ligands are expressed in the mammary epithelium, and PTCH1 is expressed in the epithelial and stromal components of the mouse mammary gland (P Visbal and T Lewis 2010).

The Hh signaling pathway has been implicated in tumorigenesis and progression of many tumor types (Fattahi, Pilehchian Langroudi et al. 2018, Raleigh and Reiter 2019). In humans, activation of the Hh pathway has been linked to cancers of the brain, lung, prostate, pancreas, colon, and breast (Lien, Klezovitch et al. 2006, Gonnissen, Isebaert et al. 2013, Armas-López, Zúñiga et al. 2017, Galdo, Natalia et al. 2019). The Hh signaling pathway has recently emerged as a therapeutic target, and inhibitors of Hh signaling, such as cyclopamine, sonidegib, and GANT-61, have gained attention (Gonnissen, Isebaert et al. 2013, Benvenuto, Masuelli et al. 2016, Galdo, Natalia et al. 2019).

In dogs, Hh signaling has been investigated for its roles in osteosarcoma and transitional cell carcinoma biology (Gustafson, Kitchell et al. 2017, Baldanza, Rogic et al. 2020); however, the relationship between Hh signaling and canine MGT has not been established. Similarities between human breast cancer and canine MGT, including genetic, histologic, and clinical elements, have been identified (Abdelmegeed and Mohammed 2018). The aim of this study was to evaluate the expression of SHH and GLI-1 in the serum and mammary tumor tissues of dogs. I hypothesized that Hh signaling elements would be more highly expressed in dogs with MGT than in normal dogs.

Materials and Methods

Patient selection

A total of 68 female or spayed female dogs were selected for this study (age: 2–17 years), which included 43 patients with MGTs and 25 healthy controls. All dogs were recruited from the Veterinary Medicine Teaching Hospital at Seoul National University (VMTH-SNU) between April 2020 and April 2022. Informed consent was obtained from the dogs' owners, and all procedures were approved by the Institutional Animal Care and Use Committees of Seoul National University (SNU-210225-3). Physical examination, complete blood count, serum biochemistry, and abdominal ultrasonography were performed to confirm that all healthy controls were normal. Patients with MGT were assessed through physical examination. Three-view thoracic radiography and abdominal ultrasonography were performed to detect their metastatic status. Complete blood count and serum biochemistry were performed before surgery. Patients with MGT underwent surgery at VMTH-SNU. They were diagnosed based on the findings of histopathological examinations conducted at IDEXX Laboratories. CT scans were performed in patients diagnosed with a malignant mammary gland tumor. Two patients with an edema or a firm and warm mammary gland mass underwent fine-needle aspiration; subsequently, mammary adenocarcinoma with necrosis and inflammation were found. In addition, the results of the fine-needle aspiration of the medial iliac lymph node showed cells with the same cell morphology as the cells in the mammary gland mass. These patients were diagnosed with inflammatory carcinoma, and surgical resection was not conducted. The patients' clinical data, tumor size, histopathological type, and metastasis were all collected.

Collection of blood samples and tissue specimens

Blood samples were collected, centrifuged at 1000 xg for 15 min to separate the serum, the collected blood samples were used to measure GLI-1 and SHH concentrations. Normal mammary gland and MGT tissue specimens were collected from dogs that underwent surgery between April 2020 and November 2021 at VMTH-SNU. Normal mammary gland was selected from the total mastectomy tissue at least one gland away from the mammary gland with one or more palpable mammary gland masses. Mammary tissue samples were cut into a volume of 5 mm3, and then were promptly frozen in liquid nitrogen and stored at −80℃ until used for western blotting. After suturing the resected area, mastectomy tissue were fixed for 24-48 hours in 10% neutral buffered formalin, and then sent to IDEXX Laboratories for analysis. A histopathological examination were performed for all palpated masses.

Measurement of serum GLI-1 and SHH

Serum GLI-1 and SHH concentrations were measured in duplicate using canine GLI-1 (Cat no: MBS7200887) and SHH (Cat no: MBS738808) ELISA kits (MyBioSource) according to the manufacturer's protocols. Briefly, 100 μL of serum and/or standards were added to the coated wells. Further, 50 μL of conjugate was added and mixed well. The plate was incubated for 1 hour at 37℃. The solution was then discarded, and the wells were washed with 1x wash solution five times. Further, 50 μL of substrate A and 50 μL of substrate B were added and incubated for 20 min at 37℃. Subsequently, 50 μL of stop solution was added, and the absorbance of each well was measured at 450 nm using a microplate reader.

Western blotting

Proteins were extracted from the normal canine mammary

gland and MGT tissue using RIPA buffer (Merck Millipore) with protease inhibitors (Sigma-Aldrich). Proteins were quantified using a BCA Protein Assay Kit (Bio-Rad), and absorbance was measured using a microplate reader at 570 nm. For denaturation, the extracted proteins (23 μg) were mixed with sodium dodecyl sulphate (SDS) loading buffer (GenDEPOT) and boiled at 100℃ for 5 min before loading. Equal amounts of protein were separated using 10% SDS polyacrylamide gel electrophoresis and transferred to a polyvinylidene difluoride membrane. The following antibodies were used in the western blotting assay: rabbit polyclonal primary antibodies against GLI-1 (1:1000, Novus Biologicals; Cat no: NB600-600) and rabbit polyclonal primary antibodies against SHH (1:500, LSBIO; Cat no: LS-C40460). The secondary antibody used was anti-rabbit IgG (1:5,000, GenDEPOT; Cat no: SA002-500). Protein expression was detected by chemiluminescence using an enhanced chemiluminescence detection reagent and visualized using an ImageQuant LAS 4000 mini biomolecular imager (GE Healthcare Bio-Sciences).

Statistical analysis

All statistical analyses were performed using SPSS software version 25.0. The relative protein expression of SHH and GLI-1 determined by western blotting were analysed using the Kruskal–Wallis test. When significant differences were observed, the Mann–Whitney U-test was performed to compare each group. Normality tests were performed using the Shapiro–Wilk test. The Tukey–Kramer method was used to compare serum SHH and GLI-1 concentrations among the three groups (healthy, benign MGT, and malignant MGT). Correlations of SHH and GLI-1 levels with clinicopathological parameters (sex, breed size, metastatic status, and histologic grade) were analysed using the Mann–Whitney U-test. The Kruskal–Wallis test was used to compare serum SHH and GLI-1 expression among histological types and tumor sizes. Kaplan-Meier survival curves were plotted and compared using the

log-rank test. All data were expressed as mean ± standard deviation. P-values < .05 were considered statistically significant.

Results

Patients

A total of 68 dogs, including 25 healthy dogs and 42 dogs with MGTs, were enrolled in this study. The clinical parameters and histological findings of the dogs are presented in Table 1. The median age was lower in the control group (6 years; range: 2–11 years) than in dogs with benign (10 years; range: 7–17 years) and malignant (11 years; range: 8–13 years) MGTs. The major breed was the Maltese. Histological classification and grade were based on the criteria described by Goldschmit et al. (Goldschmidt, Peña et al. 2011) Two patients with edema, erythema, firmness, and warmth of the mammary gland mass with distant metastasis were considered to have inflammatory carcinoma. Surgery was not performed in these patients, and histological diagnosis was not possible. Regional or distant metastases were confirmed in six patients with malignant MGT.

Table 1. Comparison of clinical parameters between healthy dogs (controls) and dogs with benign and malignant MGTs

	Control (n=25)	Benign tumor (n = 26)	Malignant tumor (n = 17)
Age, years	6 (6)	10 (2.75)	11 (2)
Sex (n)	Female (8) Spayed female (17)	Female (11) Spayed female (15)	Female (9) Spayed female (8)
Breed (n)	Maltese (7) Pomeranian (4) Golden retriever (3) Bichon frise (2) Mixed (2) Poodle (2) Chihuahua (1) Samoyed (1) Shih-tzu (1) Pekingese (1) Yorkshire terrier (1)	Maltese (12) Poodle (4) Mixed (2) Shih-tzu (2) Pomeranian (2) Bichon frise (1) Cocker spaniel (1) Jindo dog (1) Samoyed (1)	Maltese (4) Shih-tzu (3) Poodle (3) Beagle (1) Chihuahua (1) Cocker spaniel (1) Mixed (1) Pomeranian (1) Schnauzer (1) Spitz (1)
Body weight (kg)	3.44 (2.13)	3.875 (3.68)	4.60 (3.62)

Histologic classification (n)	Adenoma, simple (9) Adenoma, complex (11) Benign mixed tumor (6)	Carcinoma, simple (10) Carcinoma, complex (1) Carcinoma, mixed (3) Carcinoma, solid (1) Inflammatory carcinoma (2)
Histologic grade (n)		Grade 1 (12) Grade 2 (3) Grade 3 (0)
Metastasis (n)		6

Continuous variables are presented as median and interquartile range.

MGT, mammary gland tumor

Western blot

Western blotting was performed to confirm the expression of Hh signaling proteins in normal mammary glands and MGT tissues (Figure 1A). Relative quantitation was calculated, and comparison was based on the normal sample. SHH (Figure 1B) and GLI-1 (Figure 1C) expression levels were greater in MGT tissues than in normal tissues and were significantly higher in malignant MGTs than in benign MGTs ($P < .05$).

Serum concentrations of SHH and GLI-1 in healthy dogs and dogs with MGT

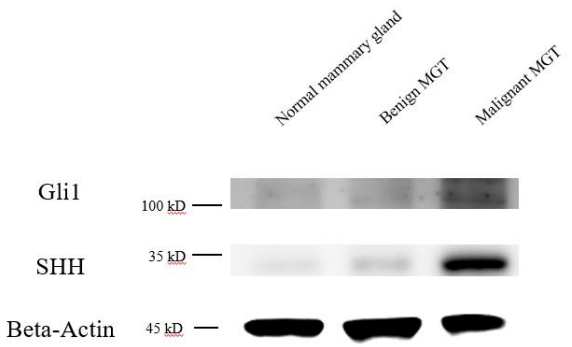
The median serum SHH and GLI-1 levels in the 25 healthy dogs were 2.38 ng/mL [95% confidence interval (CI), 1.23–3.53 ng/mL] and 17.03 ng/mL (95% CI, 10.65–23.41 ng/mL), respectively, and were significantly lower than those in the MGT group (4.53 ng/mL; 95% CI, 3.29–5.77 ng/mL and 28.19 ng/mL; 95% CI, 21.09–35.30 ng/mL, respectively; $P < .001$ and $< .001$, respectively).

To evaluate the association between Hh expression and disease severity, patients with MGT were divided into benign and malignant tumor groups (Figure 2A and 2B). The median serum SHH and GLI-1 levels of 26 dogs with benign MGTs were 3.96 ng/mL (95% CI, 2.60–5.32 ng/mL) and 24.26 ng/mL (95% CI, 15.21–33.31 ng/mL), respectively. In 17 dogs with malignant MGTs, the median serum SHH and GLI-1 levels were 5.40 ng/mL (95% CI, 3.60–7.20 ng/mL) and 34.21 ng/mL (95% CI, 25.14–43.29 ng/mL), respectively. The expression levels of SHH and GLI-1 were significantly higher in the malignant MGT group than in the benign MGT group ($P = .033$ and .006, respectively).

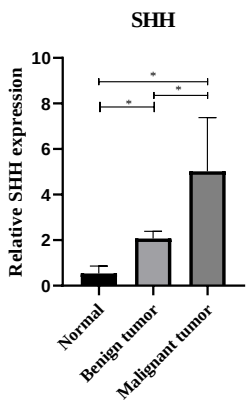
Figure 1. Comparison of SHH and GLI-1 expression in the normal mammary gland and MGT tissues

(A) SHH (35 kDa) and GLI-1 (100–140 kDa) protein levels in the normal mammary gland and benign and malignant mammary gland tissues. Beta-actin was used as the loading control. (B) Relative quantitation of SHH and (C) GLI-1 proteins were normalized to that of the control group. The mean and standard error of the mean are used to express quantitative data. (*: $p < .05$).

(A)



(B)



(C)

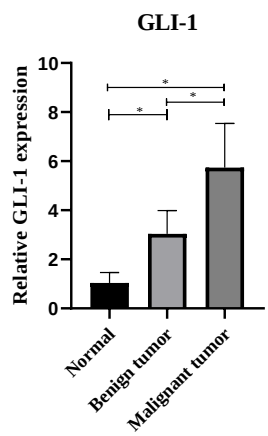
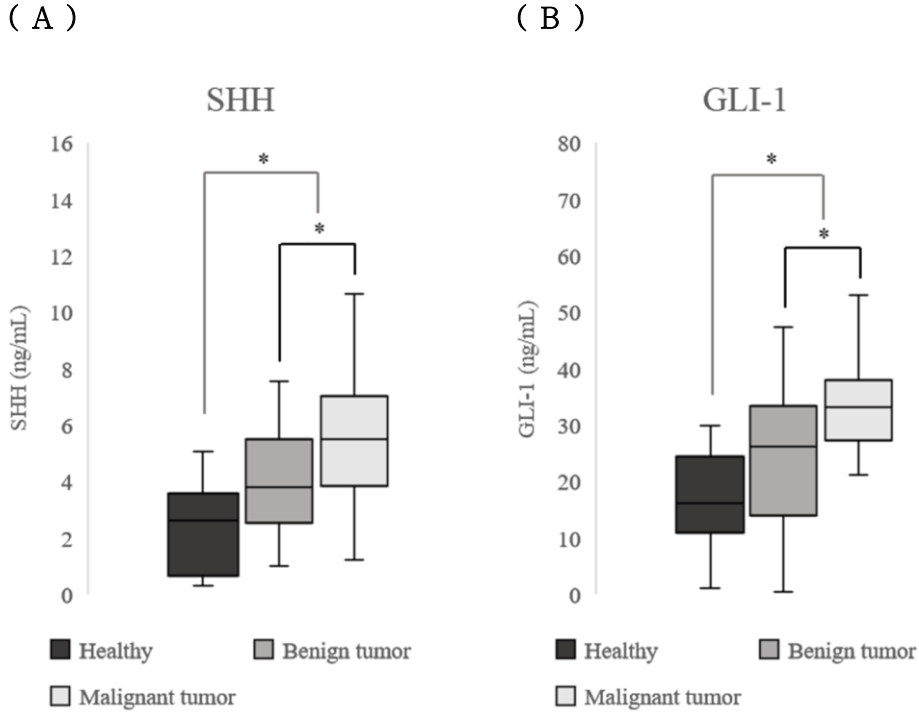


Figure 2. Serum SHH and GLI-1 levels in healthy dogs and dogs with MGT

(A) The serum SHH level was higher in dogs with MGT than in healthy dogs. (B) The serum GLI-1 level was higher in dogs with MGT than in healthy dogs. (*: $p < .05$)



Relationship between Hh signaling protein expression and clinicopathological factors

The relationship between Hh signaling protein expression and clinicopathological features is shown in Table 2. Among dogs with malignant tumors, SHH and GLI-1 expression was significantly higher in metastatic patients ($p = .01$ and .007, respectively). Dogs with regional or distant metastases were included in the metastatic group. The serum level of GLI-1 expression was correlated with histological grade ($p = .048$), but the median SHH level was not ($p = .093$). The protein expression levels showed no correlation with clinicopathological parameters such as tumor size, sex, breed size, or histological type.

Survival curve

Survival curves of 17 dogs with malignant mammary gland tumors were analyzed using a Kaplan-Meier analysis (Figure 3). Five dogs died during the study period, and all dogs died due to mammary gland tumors. The remaining 12 dogs were alive during the study. These dogs were divided into two groups based on the median SHH levels: 9 dogs with higher SHH levels and 8 dogs with lower SHH levels which were below . The analysis of the survival curves showed a statistically significant association between high levels of SHH and poor overall survival.

Table 2. Comparison of hedgehog signal expression associated with clinicopathological parameters

	Number of patients (n)	SHH (ng/mL)	P value	GLI-1 (ng/mL)	P value
Sex					
Female	20	4.17 ± 2.42		29.31 ± 10.11	
Spayed female	23	4.84 ± 1.71	.165	27.22 ± 13.40	.903
Breed size					
≤10 kg	36	4.56 ± 2.10		27.50 ± 12.47	
>10 kg	7	4.39 ± 2.08	.936	31.78 ± 8.06	.468
Tumor size					
≤3 cm	24	4.03 ± 2.01		27.28 ± 9.48	
3-5 cm	7	4.88 ± 2.83		27.43 ± 16.56	
>5 cm	12	5.32 ± 1.54	.154	30.47 ± 13.95	.534
Benign MGT					
Simple	9	4.69 ± 1.72		26.21 ± 12.23	
Complex	11	3.21 ± 1.47		22.79 ± 12.76	
Mixed	6	4.25 ± 1.91	.150	24.02 ± 10.76	.925
Malignant MGT					
Simple	10	4.32 ± 1.66		31.29 ± 7.02	
Complex	1	7.67		32.92	
mixed	3	5.41 ± 2.23	.175	29.51 ± 5.36	.925
Noninflammtory	41	4.32 ± 1.84		26.94 ± 10.67	
Inflammatory carcinoma susp.	2	8.84 ± 2.55	.020*	53.88 ± 1.02	.002*

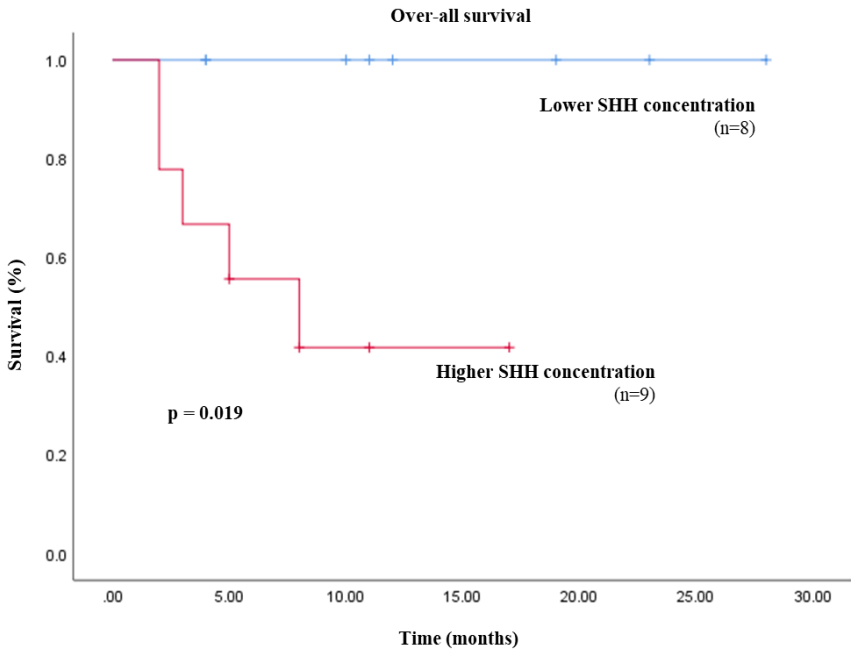
Histologic grade					
Grade 1	12	4.72 ± 2.01		30.00 ± 5.58	
Grade 2	3	5.85 ± 0.28	.448	37.94 ± 65.54	.048*
Metastasis					
Metastatic	6	7.26 ± 1.79		42.96 ± 9.81	
Non-metastatic	11	4.39 ± 1.91	.01*	29.44 ± 5.16	.007*

Continuous variables are presented as mean and SD.

SHH, sonic hedgehog GLI-1, glioma oncogene-1 MGT, mammary gland tumor

* P value <.05 indicates that the clinicopathological variable is considered to be statistically significant.

Figure 3. Kaplan-Meier survival curve of overall survival based on the sonic hedgehog (SHH) levels in 17 dogs with a malignant mammary gland tumor.



Discussion

Dysregulation of the Hh signaling pathway has been proven to be involved in the initiation and progression of various types of tumors (Yauch, Gould et al. 2008, Kasper, Jaks et al. 2009, Fattahi, Pilehchian Langroudi et al. 2018, Raleigh and Reiter 2019). Hh pathway inhibitors have been clinically developed for the treatment of several tumor types (Nguyen and Cho 2022). Recent studies have investigated the overexpression of Hh signals in human breast cancer cell lines (Song, Wang et al. 2016). In canines, several studies have been performed to determine the expression of Hh pathway mediators in different types of tumors (Gustafson, Kitchell et al. 2017, Usui, Sakurai et al. 2017, Baldanza, Rogic et al. 2020). The results of the current study suggest a relationship between Hh signaling pathway and canine MGTs.

Since SHH and GLI-1 are known to be prognostic markers in human breast cancer (Noman, Uddin et al. 2017, Wang, Yu et al. 2017) and GLI-1 is a critical transcriptional factor, I evaluated the expression of SHH and GLI-1 in normal dogs and dogs with MGT to investigate the relationship between the Hh signaling pathway and canine MGTs. Western blotting of the canine normal mammary gland and MGT tissues was performed to establish the likely sources of SHH and GLI-1 production and secretion in the serum. Previous studies have indicated that both epithelial cells and stromal fibroblasts produce Hh signaling proteins (Yauch, Gould et al. 2008, Kasper, Jaks et al. 2009). The expression of SHH and GLI-1 in mouse mammary glands and human breast has also been described previously. In addition, their expression was reported to be higher in breast cancer tissue than in normal tissue (García-Zaragoza, Pérez-Tavarez et al. 2012, Jeng, Sheen et al. 2014, Sun, Wang et al. 2014, Song, Wang et al. 2016).

The results showed that Hh expression was significantly higher in canine MGT than in normal mammary gland tissue. Malignant MGT showed higher Hh signal protein expression than benign MGT.

These results indicate the potential relationship between the Hh signaling pathway and canine MGT.

On the basis of the western blot results, I assumed that the levels of serum SHH and GLI-1 would be higher in dogs with MGT than in normal dogs. The expression was significantly higher in dogs with malignant MGT than in those with benign MGT, as well as in dogs with MGT than in normal dogs. Increased SHH and GLI-1 protein levels in the serum of patients with MGT suggest that these factors may serve as biomarkers.

Correlation analyses were performed between the expression of Hh signal proteins (SHH and GLI-1) and clinicopathological parameters, which are known risk or prognostic factors (Misdorp and Hart 1976, Vail, Thamm et al. 2019). Patients with inflammatory carcinoma have significantly higher SHH and GLI-1 levels than those with non-inflammatory MGT because patients with inflammatory carcinoma have signs of systemic illness and distant metastases (Vail, Thamm et al. 2019). In this study, serum GLI-1 was positively correlated with histological grade; however, the correlation between SHH and histological grade was not statistically significant. In general, canonical activation of the Hh signaling pathway, which is initiated by Hh ligand binding, occurs mainly in cancers and cancer stem cells. In the non-canonical Hh signaling pathway, GLI-1 activity is induced independently of the presence of the Hh ligand (Lauth and Toftgård 2007). It has been reported that several mechanisms of non-canonical activation may co-exist (Galdo, Natalia et al. 2019, Pietrobono, Gagliardi et al. 2019, Nguyen and Cho 2022). There is a lack of information on the role of non-canonical Hh signaling in canine MGT, and the results of serum GLI-1 and SHH levels based on the histologic grade suggest a relationship between the non-canonical pathway of Hh signaling and canine MGT. Further studies are required to confirm this hypothesis. Serum SHH showed a positive correlation with metastatic status. These data are consistent with previous reports in humans (Noman, Uddin et al. 2017). Serum GLI-1 levels and metastatic status were also statistically correlated.

These findings demonstrated that higher serum SHH concentration patients showed poor overall survival than lower serum SHH concentration patients ($p = .019$). In human breast cancer, high serum SHH levels were associated with poor overall survival (Noman, Uddin et al. 2017). The results of this study imply the possibility of SHH as a valuable prognostic marker in canine mammary gland tumors.

This study has several limitations. First, the serum sample sizes of normal dogs and dogs with MGT were relatively small. The correlations based on tumor grade and molecular classification were limited. A larger sample size would help to obtain reliable results and a multi-perspective analysis. Second, the presence of Hh signaling proteins (SHH and GLI-1) in normal mammary glands and MGTs was confirmed only at the protein level. Further investigations, such as reverse transcription–polymerase chain reaction to identify its presence at the mRNA level and immunohistochemistry to confirm protein location, are needed to address these issues. Also, estrogen expression has been shown to be correlated with GLI-1 expression, and the estrogen receptor regulates non-canonical hedgehog signaling (Sun, Wang et al. 2014). Immunohistochemistry of mammary gland tissue for the estrogen receptor and evaluation of the patient's serum hormonal levels would advance the understanding of the Hh signaling pathway in patients with MGT. In addition, only a portion of the Hh signaling proteins (SHH and GLI-1) was observed. Examining the expression of other Hh signaling pathway factors would improve therapeutic options for other Hh pathway inhibitors.

In conclusion, the results suggest that the Hh signaling pathway is associated with canine MGT. Hh signal protein expression in the serum was higher according to the histologic classification and metastatic status. This study opens up the possibility of using the Hh signaling pathway as a new biomarker and therapeutic target in canine MGT patients. Further studies are needed to understand the specific mechanism by which the Hh signaling pathway promotes the development of canine MGTs.

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국문 초록

유선종양 개 혈청에서의 헤지호그 신호 단백질에 대한 비교

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Hedgehog signaling pathway (헤지호그 신호) 는 다양한 종류의 종양형성과 진행에 연관이 되어 있다고 알려져 있어, 최근 새로운 치료 타겟 및 biomarker 로써 주목 받고 있다. 개의 경우 TCC, osteosarcoma 와 헤지호그 신호의 연관성에 대한 연구가 이루어졌으나, 유선종양과 헤지호그 신호의 연관성에 대해서는 알려진 바가 없다. 이 연구는 헤지호그 신호 단백질 중, sonic hedgehog(SHH) 와 glioma oncogene-1(GLI-1)가 유선종양 환자의 혈청에서 건강한 개체보다 더 많이 발현 되는 지를 확인하는 것을 목표로 했다.

SHH 와 GLI-1 단백질이 유선종양 조직에서 정상 유선 조직보다 더 유의미하게 발현 되었으며, 양성으로 진단 된 유선종양 조직보다 악성으로 진단 된 유선종양 조직에서 더 높게 발현되는 것을 확인할 수 있었다. 또한 건강한 개체보다 유선종양 개의 혈청에서 SHH, GLI-1 단백질 농도가 더 높은 것을 확인할 수 있었다. 혈청 SHH 의 경우, 전이 여부에 따라 유의미한 차이를 보였으며(P

= .01), 혈청 GLI-1 은 전이 여부(P = .007), 조직학적인 단계(P = .048)에 따라 유의미하게 높은 농도를 보였다.

유선종양의 조직학적인 종류(양성 혹은 악성) 및 전이 상태에 따라 개 유선종양 환자 혈청에서의 헤지호그 신호 발현이 유의미하게 증가하는 것을 확인할 수 있었다. 결론적으로 이 연구 결과는 개의 유선종양과 헤지호그 신호간의 관련성을 나타내며, 개 유선종양에서 헤지호그 신호의 새로운 치료 타겟 및 바이오 마커로서 활용될 가능성을 제시하였다.

주요어: 헤지호그 신호, 개, 혈청, 유선종양

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