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RESEARCH ARTICLE

Distribution of Some Heat Shock Proteins in the Tongue Tissues of Sheep of Different Ages

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Abstract

Heat shock proteins are molecular chaperones that regulate and modulate a multitude of cellular and physiological processes. This study was designed to determine the immunoexpression of HSP27 and HSP90 in tongue tissues throughout the development of sheep. Tongue tissues were collected from sheep aged 6-12 months (G1,n:6), 1-2 years (G2,n:6), and 3-5 years (G3,n:6). Immunohistochemical staining was performed after the tissue samples were subjected to routine histological procedures. Immunoreactivity for HSP27 and HSP90 was not observed in peripheral nerves, serous Von Ebner's glands, and mucous glands. While HSP27 immunoreactivity was not observed in the gland duct epithelium, HSP90 immunoreactivity was detected. HSP27 and HSP90 immunoreactivity was seen in the epithelial layer, skeletal muscle cells, vascular endothelium, and vascular smooth muscle cells. There were no statistically significant differences in HSP90 immunoreactivity in the gland duct epithelium, epithelial layer, blood vessels, and skeletal muscle cells throughout the development of the sheep ($p>0.05$). While HSP27 immunoreactivity in blood vessels and the epithelial layer was not statistically changed between groups ($p>0.05$), HSP27 immunoreactivity in skeletal muscle cells was statistically higher in G1 compared to G3 ($p<0.01$). The results of this study demonstrated that HSP27 and HSP90 were expressed in the luminal epithelium, blood vessels, and skeletal muscle cells of the tongue tissues throughout the development of the sheep. This study shows that HSP27 and HSP90 are essential for sheep tongue development and play critical roles in cellular events.

Key Words: Heat shock protein, HSP27, HSP90, immunohistochemistry, sheep, tongue

Farklı Yaşlardaki Koyunların Dil Dokusunda Bazı Isı Şok Proteinlerinin Dağılımı

Öz

Isı şok proteinleri çok sayıda hücresel ve fizyolojik süreci düzenleyen ve modüle eden moleküler şaperonlardır. Bu çalışma, koyunların gelişimi boyunca dil dokularında HSP27 ve HSP90' nın immüneksp-resyonunu belirlemek için tasarlandı. 6-12 aylık (G1,n:6), 1-2 yaşındaki (G2,n:6) ve 3-5 yaşındaki (G3,n:6) koyunlardan dil dokuları toplandı. Doku örnekleri rutin histolojik işlemlere tabi tutulduktan sonra immünohistokimyasal boyama yapıldı. Periferik sinirlerde, seröz Von Ebner bezlerinde ve müköz bezlerinde HSP27 ve HSP90 immünoreaktivitesi gözlenmedi. Kanal epitelinde HSP27 immünoreaktivitesi görülmezken, HSP90 immünoreaktivitesi tespit edildi. Epitel katmanda, iskelet kası hücreleri, damar endoteli ve damar düz kas hücrelerinde HSP27 ve HSP90 immünoreaktivitesi görüldü. Epitel katman, iskelet kası hücreleri, kanal epiteli ve kan damarlarındaki HSP90 immünoreaktivitesinde koyunların gelişimi boyunca istatistiksel olarak anlamlı bir farklılık saptanmadı ($p>0.05$). Kan damarları ve epitel katmandaki HSP27 immünoreaktivitesi gruplar arasında istatistiksel olarak değişmezken ($p>0.05$), iskelet kası hücrelerindeki HSP27 immünoreaktivitesi G3'e kıyasla G1'de istatistiksel olarak daha yüksekti ($p<0.01$). Bu çalışmanın bulguları HSP27 ve HSP90'nın koyunların gelişimi boyunca dil dokularının epitel katmanından, kan damarları ve iskelet kası hücrelerinden eksprese olduğunu göstermiştir. Bu çalışma, HSP27 ve HSP90'nın koyunların dil gelişimi için gerekli olduğunu ve hücresel olaylarda kritik roller oynadığını göstermektedir.

Anahtar Kelimeler: Dil, HSP27, HSP90, ısı şoku proteini, immünohistokimya, koyun

INTRODUCTION

The manner in which vertebrates are fed and their dietary habits are significant factors in determining their capacity to adapt to their environment. The tongue, in conjunction with other oral cavity organs, plays a pivotal role in the process of feeding. The structural differences observed in the tongue are indicative of the specific food sources and the particular habitat of each species of mammal (1,2).

The tongue is covered with stratified squamous keratinized epithelium. The keratinized stratified squamous epithelium is divided into several layers: the stratum basale, the stratum spinosum, the stratum granulosum, and the stratum corneum (3). The tongue's mucosa comprises various papillary systems that serve gustatory and mechanical functions (1,4). The majority of the mammalian tongue is composed of longitudinal, transverse, and vertical skeletal muscle cells (3). The submucosa and connective tissue between skeletal muscle cells contain serous Von Ebner's, mucous, and seromucous lingual glands. The connective tissue beneath the epithelium includes adipose tissue, blood vessels, and nerve plexuses (5).

Heat shock proteins (HSPs), also known as molecular chaperones, are highly conserved proteins (6). These proteins are essential for the normal functioning of cells and play a significant role in protecting cells against damage in stressful conditions (7,8). HSPs are overexpressed in a variety of environmental conditions, including temperature changes, oxidants, ethanol, radiation, viral infections, heavy metal ions, and anoxia (8). HSPs are known to perform several cell-protective functions, including protein assembly and disassembly, interaction with surface receptors, and antigen presentation (6,7). HSPs are categorized according to their molecular weight and function. This classification includes the small HSPs, HSP40, HSP60, HSP70, HSP90, and HSP110 (7,9,10).

HSP27 is a small molecular weight HSP family member and an ATP-independent chaperone (11,12). HSP27 prevents the aggregation of misfolded proteins and assists in correctly folding proteins. HSP27 is involved in cellular functions such as proliferation, differentiation, migration, and signal transduction (11). HSP27 is associated with several intermediate filament networks. HSP27 regulates apoptosis by interacting with key components of the apoptotic signaling pathway. HSP27 is associated with actin and regulates the polymerization of actin (12).

HSP90 forms 1-2% of cellular protein under physiological conditions. HSP90 is an ATP-dependent molecular chaperone that regulates the activation, late maturation, and stability of a variety of proteins. HSP90 interacts with nuclear or cytoplasmic proteins, including transforming or regulatory tyrosine kinases, some serine/threonine kinases, transcription factors, cytoskeletal proteins, or calmodulin. HSP90 is involved in fundamental cellular processes and regulatory pathways such as apoptosis, cell signaling, cell cycle control, and cell viability by interacting with other proteins and co-chaperones in the cells (13). HSP90 plays a role in antigen presentation, and activation of lymphocytes, macrophages, and dendritic cells (14).

The tongue is an important organ that can provide information about the general health status of animals. Therefore, studies on the tongue of sheep are of great importance both for applied veterinary medicine and animal health and for basic biological research. Changes in the expression of molecular factors in tongue tissue can be used to understand sheep's responses to temperature changes, malnutrition, or other stress factors. Knowing the expression patterns of molecular factors in the tongue tissue, which undergoes structural and morphological changes during the developmental process of sheep, may contribute to the understanding of the functional properties and adaptation mechanisms of the tongue. HSP27 and HSP90 play important roles in metabolic events in cells under normal physiological conditions, while under various stress conditions, the expression of these proteins in cells is increased and they play important roles in cellular repair and protection mechanisms. There are a limited number of studies on the expression of HSP27 and HSP90 in tongue tissue. Therefore, this study aimed to show the expression of HSP27 and HSP90 in the tongue tissues of sheep of different ages by immunohistochemistry.

MATERIAL AND METHODS

Animal Material and Tissue Processing

The tissue material used in this study was obtained from sheep that were brought to the abattoirs in the province of Siirt for slaughter. Tongue tissues from a total of 18 sheep aged 6-12 months (G1,n=6), 1-2 years (G2,n=6), and 3-5 years (G3,n=6) were used as tissue material in the study. Small pieces of tissue taken from the tongue of each animal were fixed in 10% formaldehyde (pH=6.9-7.1) for 24 hours at room temperature. Tissue samples were embedded in the paraffin after routine histological processing. Tissue blocks were cut at a thickness of 5 microns. Sections were transferred to poly-L-lysine-coated slides.

Immunohistochemical Staining

Immunohistochemical staining was performed using the streptavidin-biotin-peroxidase complex (Strept-ABC) to determine the immunoreactivities of HSP27 and HSP90 in sheep tongue tissues of different ages. The sections were passed through the xylol-alcohol series. Afterward, they were placed in citrate buffer (pH=6) and subjected to boiling in a microwave oven at 600W for 20 minutes to facilitate antigen retrieval. To inhibit the endogenous peroxidase activity, the sections were incubated in 3% H_2O_2 in PBS for 20 min. The sections were encircled with a hydrophobic PAP pen and maintained in a blocking solution (Large Volume Ultra V Blok, TA-125-UB, Thermo Fisher Scientific) for 10 minutes to prevent non-specific antigenic binding. Afterward, the sections were incubated with HSP27 (Santa Cruz Biotechnology, sc-13132, dilution: 1/150) and HSP90 (Santa Cruz Biotechnology, sc-13119, dilution:1/200) primary antibodies at 4°C overnight. The next day, a biotinylated secondary antibody (Biotinylated Goat Anti-Polyvalent, Thermo Fisher Scientific, TP-125-BN) was applied to the sections for 30 minutes. Then, they were kept with enzyme-conjugated streptavidin solution (Streptavidin Peroxidase, Thermo Fisher Scientific, TS-125-HR) for 30 minutes. To visualize the immunostaining, the sections were incubated in 3,3' diaminobenzidine tetrahydrochloride (Large Volume DAB Substrate System, Thermo Fisher Scientific, TA-125-HD) for 1 minute. Nuc-

lear counterstaining was conducted using Harris' hematoxylin. The sections were then subjected to the alcohol-xylol series and covered with Entellan. To ascertain the specificity of the results, negative control sections were performed by the same procedure, but treated with PBS in place of the primary antibody. The prepared sections were examined under a light microscope (DM750, Leica) connected to a digital camera (MC170, Leica).

Semiquantitative Evaluation

The immunostaining for HSP27 and HSP90 was evaluated semiquantitatively based on intensity scores (ISs) (15). The grading of the IS was based on a four-point scale: 0, no immunoreaction; 1, weak immunoreaction; 2, moderate immunoreaction; and 3, strong immunoreaction. The immunostaining of HSP27 and HSP90 in the sheep tongues was examined microscopically at 10x, 20x, and 40x objective magnification. Ten areas per section were randomly selected for evaluation and the average of these results was taken as a value.

Statistical Analysis

Statistical analysis of the data was performed using Minitab® (v21.4.1). To assess the normal distribution of the data, the Anderson-Darling normality test was performed. Non-parametric tests were preferred as the data were not normally distributed. The Kruskal-Wallis test was used to evaluate HSP90 and HSP27 immunoreactivity between age groups in sheep tongue tissue. The statistical significance level was evaluated as $p<0.05$.

RESULTS

HSP27

During the development of the sheep, no immunoreactivity for HSP27 was observed in the peripheral nerves (Figure 1d), gland duct epithelium (Figure 1e), serous Von Ebner's glands (Figure 1f), and mucous glands (Figure 1e) in the tongue tissues. However, skeletal muscle cells (Figure 1c,d), vascular endothelium, and vascular smooth muscle cells (Figure 1c) exhibited cytoplasmic HSP27 immunostaining. In addition, cytoplasmic HSP27 immunoreactivity was detected in the stratum basale, stratum spinosum, and stratum granulosum of the epithelial layer, excluding the stratum corneum. In the stratum basale, some cells showed negative HSP27 immunoreactivity, while some cells showed very weak HSP27 immunoreactivity. HSP27 immunostaining in the epithelial layer gradually increased from stratum basale to granulosum (Figure 1b).

There was no statistically significant difference in HSP27 immunoreactivity in the luminal epithelium, vascular endothelium, and vascular smooth muscle cells throughout the development of the sheep ($p>0.05$) (Table 1). However, HSP27 immunostaining in skeletal muscle cells was statistically higher in G1 (Figure 1c) compared to G3 (Figure 1d) ($p<0.01$) (Table 1).

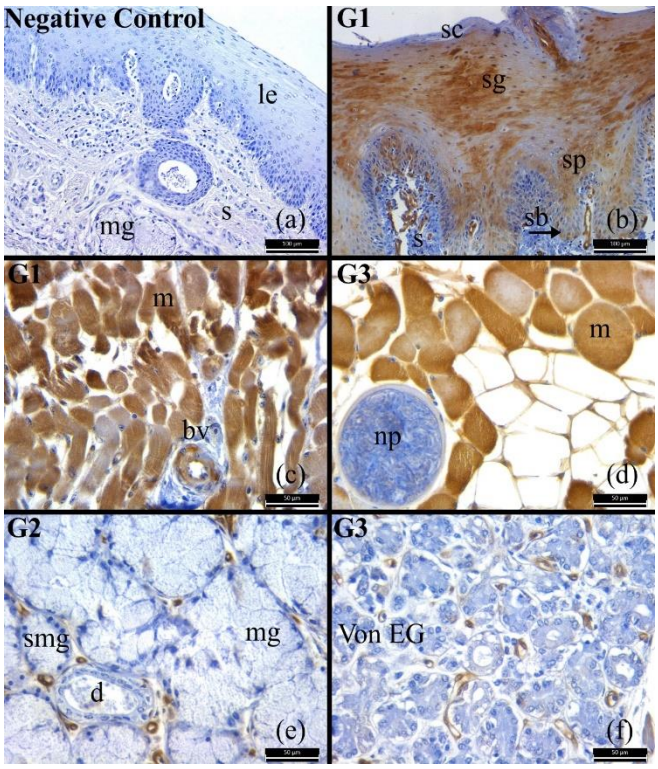

Figure 1. Immunostaining of HSP27 in the tongues of sheep aged 6-12 months (G1), 1-2 years (G2), and 3-5 years (G3). bv: blood vessel, d: excretory ducts, le: luminal epithelium, m: skeletal muscle cell, mg: mucous gland, np: nerve plexus, s: stroma, sb: stratum basale, sc: stratum corneum, sg: stratum granulosum, smg: seromucous gland, sp: stratum spinosum, Von EG: serous von Ebner's gland. Bar: a-b: 100 µm; c-f: 50 µm.

Table 1. Intensity scores for HSP27 and HSP90 expression in the tongues of sheep aged 6-12 months (G1), 1-2 years (G2), and 3-5 years (G3)

	G1	G2	G3	p-value
HSP27				
Luminal epithelium	2.00^{a}	1.50^{a}	1.00^{a}	>0.05
Skeletal muscle cell	3.00^{a}	2.50^{ab}	2.00^{b}	0.003**
Mucous gland	0	0	0	-
Serous Von Ebner's gland	0	0	0	-
Gland duct epithelium	0	0	0	-
Peripheral nerve	0	0	0	-
Blood vessel endothelium	3.00^{a}	3.00^{a}	3.00^{a}	>0.05
Blood vessel smooth muscle cell	3.00^{a}	3.00^{a}	3.00^{a}	>0.05
HSP90				
Luminal epithelium	2.00^{a}	1.50^{a}	2.00^{a}	>0.05
Skeletal muscle cell	2.50^{a}	3.00^{a}	3.00^{a}	>0.05
Mucous gland	0	0	0	-
Serous Von Ebner's gland	0	0	0	-
Gland duct epithelium	1.50^{a}	1.00^{a}	1.00^{a}	>0.05
Peripheral nerve	0	0	0	-
Blood vessel endothelium	1.00^{a}	1.00^{a}	1.00^{a}	>0.05
Blood vessel smooth muscle cell	1.00^{a}	1.00^{a}	1.00^{a}	>0.05

**p<0.01; a, b; different letters indicate that the differences between the median values of the age groups are significant.

HSP90

No immunoreactivity for HSP90 was observed in the peripheral nerves (Figure 2c), serous Von Ebner's glands (Figure 2d), and mucous glands (Figure 2e) in the tongue tissues of sheep of different ages. In the luminal epithelium of the sheep tongues, HSP90 positivity was detected in the cytoplasm of epithelial cells in the stratum basale, stratum spinosum, and stratum granulosum excluding the stratum corneum (Figure 2b). Some gland duct epithelium cells showed nuclear HSP90 positivity (Figure 2e). In addition, cytoplasmic HSP90 immunoreactivity was found in skeletal muscle cells (Figure 2c,f), vascular endothelium, and vascular smooth muscle cells (Figure 2f).

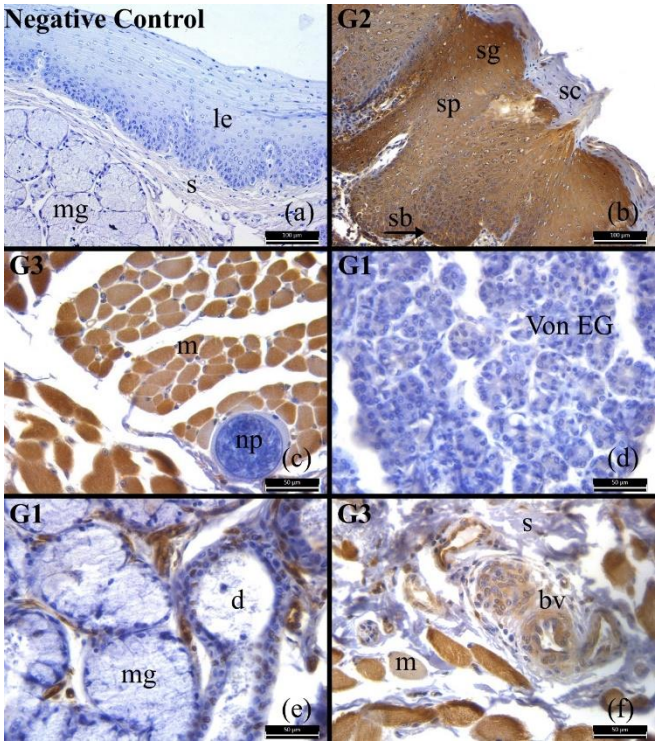

Figure 2. Immunostaining of HSP90 in the tongues of sheep aged 6-12 months (G1), 1-2 years (G2), and 3-5 years (G3). bv: blood vessel, d: excretory ducts, le: luminal epithelium, m: skeletal muscle cell, mg: mucous gland, np: nerve plexus, s: stroma, sb: stratum basale, sc: stratum corneum, sg: stratum granulosum, sp: stratum spinosum, Von EG: serous von Ebner's gland. Bar: a-b: 100 µm; c-f: 50 µm.

HSP90 immunoreactivity in the epithelial layer, skeletal muscle cells, gland duct epithelium, vascular endothelium, and vascular smooth muscle cells did not change statistically throughout the development of the sheep ($p>0.05$) (Table 1).

DISCUSSION AND CONCLUSION

HSPs play a critical role in maintaining cellular homeostasis, promoting cell survival under stress conditions, and modulating various cellular processes that are essential for normal cell function and organismal health. It is very important to know the localization and expression in tissues of these proteins, which are very important for organisms. Therefore, in this study, the localization and expression of HSP27 and HSP90 were demonstrated in the tongue tissue, which is an important organ of the digestive system, throughout the developmental process of sheep.

Tekkesin et al. (16) reported that HSP27 was not expressed in healthy human tongue. In normal human skin, Wilson et al. (17) determined that HSP27 was expressed in the basal and suprabasal layers of the epidermis. In a study carried out in rats, Zheng et al. (18) showed that HSP27 was present in the cytoplasm of the epithelial cells of the esophagus. In normal mouse epidermis, Laplante et al. (19) indicated that HSP27 had a suprabasal pattern of expression, but the stratum corneum was not labeled. In a study conducted on normal human skin, Gandour-Edwards et al. (20) reported that HSP27 was not expressed in the basal layer and stratum corneum layer of the epidermis, but that HSP27 expression in the suprabasal layer gradually increased from the stratum spinosum to the stratum granulosum. In the canine epidermis, Romanucci et al. (21) observed that HSP27 was expressed in the stratum spinosum and granulosum, whereas the stratum basale exhibited negative or only weakly positive immunoreactivity. The researchers also reported that the intensity of immunolabeling in the upper layers showed a gradual increase from positive to strongly positive in the stratum granulosum, while the stratum corneum was negative. In the present study, a similar HSP27 immunostaining pattern was detected in the epithelial layer of tongue tissues throughout the development of sheep. HSP27 involves many cellular processes, including signal transduction, differentiation, proliferation, and cellular movements. It plays a role in keratinocyte differentiation and epidermis development and acts as a chaperone for keratinization (11,20,21). Based on this information and the results obtained, it can be said that HSP27 plays a role in epithelial cell proliferation and keratinocyte differentiation in sheep tongues.

In normal human skin, Wilson et al. (17) demonstrated that HSP90 was present in the basal and suprabasal layers of the epidermis. In a study on the normal mouse epidermis, Laplante et al. (19) reported that HSP90 was mainly present in the high suprabasal cells, with basal and low suprabasal cells showing very weak labeling. Yang et al. (22) detected that HSP90 was present in the epidermis of yak skin. Similarly, HSP90 was shown to be expressed in the epithelial layer of the sheep tongues in this study. It has been observed that HSP90 immunoreactivity in the epithelial layer, like HSP27, did not change statistically throughout the development of the sheep. However, in contrast to HSP27, the HSP90 immunostaining pattern in the epithelial layer was homogeneous. These findings suggest that HSP90 is constitutively expressed in the epithelial layer, excluding the stratum corneum, throughout the developmental process of the sheep and that HSP90 functions as a chaperone protein to ensure the homeostasis of epithelial cells.

HSP27 is an actin-associated protein that regulates the polymerization of actin in the cells (12). HSP27 is expressed in the tongue and cardiac muscles during mouse embryogenesis (23) and plays a critical role in developing cardiac and skeletal muscle tissues (24). In a study in rats (25), HSP27 immunoreactivity was found in cardiac and skeletal muscle cells, as well as in the smooth muscle cells of blood vessels and hollow organs. In adult vertebrate muscle tissue, HSP27 is expressed at high levels in slow-twitch skeletal and cardiac muscle, with lower but still significant expression in fast-twitch skeletal muscle (26-28). Sun et al. (29) detected that

HSP 27 showed a down-regulation of expression in the tibialis anterior muscle of the rat during postnatal growth and development. Similarly, the present study showed that HSP27 was expressed in skeletal muscle cells in the sheep tongues and that HSP27 immunoreactivity statistically decreased during sheep development. After birth, skeletal muscle cells continue to grow and develop into adulthood. The growth and development of skeletal muscle cells involve many different processes that are controlled by many factors, including growth factors, hormones, kinases, and transcription factors. Although the growth and development of skeletal muscle cells have been the focus of many researchers, relatively little is known about these events (29). The decrease in HSP27 immunoreactivity in the skeletal muscle cells of the tongue due to the development of sheep shows that HSP27 plays a role in important events during the growth and development of skeletal muscle cells. However, more detailed studies need to be conducted on this subject.

HSP90 is expressed in a wide variety of tissues, including skeletal muscle cells, where it functions as a myosin chaperone (30). HSP90 participates in the modification of myosin in myofibrils (31). Moreover, HSP90 plays a pivotal role in the correct folding of the motor domain, which is also referred to as myosin subfragment-1 (S1) in skeletal muscle cells (30,32). Srikakulam and Winkelmann (30) suggest that HSP90 is involved in the initial folding of striated muscle myosin. Bornman et al. (33) detected moderate levels of HSP90 immunoreactivity in the sarcoplasm and nucleus of mature muscle cells. In this study, HSP90 positivity was detected in the skeletal muscle cells in the tongue of sheep and it was found that HSP90 reactivity in skeletal muscle cells did not change statistically throughout the development of the sheep. These results indicate that HSP90 is constitutively expressed in the skeletal muscle cells of the tongue throughout the developmental process of sheep.

In humans, Basset et al. (34) reported that HSP27 and HSP90 were present in the acini and ducts of embryonic salivary glands, but in adult glands, HSP27 was only present in the ducts and HSP90 was completely absent. In a study conducted on adult human salivary glands, Vanmuylder et al. (35) showed positive expression of HSP27 and HSP90 in epithelial cells of striated and excretory ducts, whereas HSP27 and HSP90 were not expressed in acinic cells. Takahashi-Horiuchi et al. (36) indicate that HSP27 was only expressed in the vascular endothelium, nerve fibers, and parts of the interlobular duct in normal rat submandibular glands. In this study, no immunostaining for HSP27 and HSP90 was observed in the serous Von Ebner's glands, mucous glands, and nerve plexuses of the sheep tongues. However, in contrast to HSP27, HSP90 immunostaining was seen in gland ductal epithelium cells.

In the rat uterus during the involution process, Liman (37) reported that blood vessels had moderate cytoplasmic and strong nuclear HSP90 immunoreactivity in endothelial and smooth muscle cells. Zheng et al. (18) found that HSP27 was present in smooth muscle cells and vascular endothelial cells in the esophagus of rats. Bao et al. (38) indicated that HSP27 and HSP90 were consistently present in the endothelium of the glomerular capillaries in pigs. Similarly, in this study, constitutive expression of HSP27 and HSP90 were determined in the vascular endothelium and vascular smooth muscle cells of the tongue throughout the developmental process of sheep.

In conclusion, this study demonstrated the localization and expression of HSP27 and HSP90 in tongue tissue throughout the developmental process of sheep. In all study groups, HSP27 and HSP90 were not expressed in peripheral nerves, serous Von Ebner's glands, and mucous glands, but were expressed in the skeletal muscle cells, epithelial layer, vascular endothelium, and vascular smooth muscle cells. Throughout the development of the sheep, HSP27 immunoreactivity decreased in skeletal muscle cells, whereas HSP90 immunoreactivity did not change. In the luminal epithelium of the tongue of sheep of all ages, while HSP90 showed a homogeneous staining pattern, HSP27 immunostaining gradually increased from the basal layer to the granulosum. HSP27 and HSP90 immunoreactivity in the luminal epithelium and blood vessels did not change throughout the developmental process of the sheep. In addition, in contrast to HSP27, HSP90 immunoreactivity was detected in the gland duct epithelium.

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CONFLICTS OF INTEREST

The author declares that she has no conflict of interest.

AUTHOR CONTRIBUTIONS

All processes, such as the design of the study, tissue collection, laboratory procedures, data analysis, preparation of the original draft of the article and the revision process, were conducted by Banu KANDİL.

ETHICAL STATEMENT

This study was approved by Siirt University Local Ethics Committee for Animal Experiments (File no: 2024/11, Decision no: 2024/03/11).

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