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Distribution of epidermal growth factor receptor, bone morphogenetic protein-2, and p53 in kidney of healthy newborn, adult and old highland-plateau yaks

T. Wang, Y. Cui, P. Liu, J. He, Q. Zhang

Faculty of Veterinary Medicine, Gansu Agricultural University, Lanzhou, China

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Background: *Kidney has long been thought to be a body's largest organ of elimination for maintaining acid-base balance. In recent years, the research on kidneys has mainly focused on the structural characteristics of the kidney of single age group animals. In this paper we used histological and immunohistochemical methods to observe and compare the structure characteristics of yak kidney and the expression of epidermal growth factor receptor (EGFR), bone morphogenetic protein-2 (BMP-2) and p53 in the kidney of yaks of three different age groups. The aim of the study was to investigate histological characteristics of age-related changes in the kidney of yak and expression and localisation of kidney-related factors.*
Materials and methods: *Fifteen healthy male and female yaks from highland plateaus (three groups: newborn, adult and old yaks, n = 5 per group). Histological methods were used to compare the relevant characteristics of the kidney of yaks. The immunohistochemistry method was used to observe the expression and localisation of EGFR, BMP-2, and p53 of the kidney of different ages, and the optical density value was measured and analysed by using image analysis software.*
Results: *This is an overall observation of the kidney tissue section, which includes the surface of the renal capsule and the internal parenchyma. In the renal parenchyma, there are renal corpuscles, renal tubules. The internal substance included cortex and medulla, which were bounded by the arched artery. In the cortex, there were renal corpuscles, convoluted part of renal tubules (proximal convoluted tubule and distal convoluted tubule) and collecting tubules. The medulla included straight parts of renal tubules (proximal straight tubule and distal straight tubule), thin segments and collecting tubules. It was observed that the organisational structure of the kidney of yaks did not change with age, but the degree of development of the internal structure (glomeruli, renal tubules and collecting tubules) of the kidney changed with age. Immunohistochemical results demonstrated that EGFR and BMP-2-positive reaction in the newborn group was mainly distributed in the proximal tubule epithelial cells, and widely distributed in the adult and old groups. However, the p53-positive reaction was widely distributed in the newborn, adult and old groups.*
Conclusions: *The results revealed that the kidney structure tended to be completed with age, and the function of the kidney gradually improved. EGFR and BMP-2 had the effect of promoting kidney development. However, p53 had been widely distributed in the newborn kidney of the yaks. It is suggested that p53 had been involved in cell migration and metabolic differentiation and self-renewal in the new stage.* (Folia Morphol 2019; 78, 1: 114–123)

Key words: bone morphogenetic protein-2, epidermal growth factor receptor, kidney, tumour suppressive gene p53, yak

Address for correspondence: Dr. Y. Cui, College of Veterinary Medicine, Gansu Agricultural University, Lanzhou 730070, China, tel: +86 0931-7631229, e-mail: cuiyan369@sina.com

INTRODUCTION

As the largest excretory organ in the body, the kidney plays a vital role in regulating metabolism and acid-base balance in the body. The existing research on kidneys has been mainly focused on the structural characteristics of the kidney of single age group animals such as dog, rat, mouse and, Bactrian Camels [2, 36]. However, studies on the kidney of different ages were found only in mouse [37] and human [18]. So far, since the yak (*Bos grunniens*) is a species that reproduced in the alpine zone of the Qinghai-Tibet Plateau and the study focused on respiratory system [38], circulatory system [13], immune system [40] and reproductive system [25], including organisational structure, ultrastructure and some factors. There is no detailed information from histological studies on the impact of kidney-related factors and age on yak kidneys.

Epidermal growth factor receptor (EGFR) is a kind of glycoprotein, which belongs to the tyrosine kinase receptor, and its molecular weight is 170 kDa. EGFR is located on the surface of the cell membrane and is activated by binding to a ligand, including EGF and transforming growth factor (TGF)-alpha [8]. EGFR plays an important role in physiological processes such as growth, proliferation, and differentiation of cells [30]. Studies have shown that EGFR is widely distributed in the kidney [33]. The distribution of EGFR in human kidney tissue is the same as that in mouse and rat kidney tissue [11]. It has recently been reported that activation of EGFR leads to phosphorylation of Smad 1, the downstream target of bone morphogenetic protein, by a mitogen-activated protein kinase (MAPK)-dependent mechanism [22].

Bone morphogenetic proteins (BMPs) are a group of osteogenic factors, and structurally, BMPs are highly homologous to TGF-beta family of proteins [14, 27]. BMPs exert their biologic effects via type II and type I serine-threonine kinase receptors [1, 14, 27]. In the current study, BMPs have been shown to play an important role in the development of gut, heart, skin, teeth, lung and kidney [16]. High-affinity binding sites for BMP-2, a specific member of this family of proteins, had been identified in different cells and tissues including the kidney [19]. BMP-2 may regulate both kidney development and adult tissue maintenance [35].

The tumour suppressive gene p53 is widely known as a key regulator of apoptosis by acting both as an active component of the apoptosis cascade and as a transcription factor [3]. p53 is one of the key regulatory genes activated during kidney development which induces cell cycle arrest or apoptosis to maintain genome integrity [15]. A previous study demonstrated that p53 phosphorylation is involved in the development of the kidney [5]. Intrauterine growth restriction (IUGR) model established by uterine artery ligation observed a parallel increase in p53 protein level and apoptosis in the kidneys of IUGR rats after birth [3]. p53 signalling is highly complex and involved in kidney development through multiple pathways [12].

In this study, we used histological and immunohistochemical methods to observe and compare the structure characteristics of yak kidney and the expression of EGFR, BMP-2 and p53 in the kidney of yaks of three different age groups. The purpose of this study was to explore the age-related changes of healthy plateau yaks and the expression of renal--related factors in the kidney yaks of different ages. It laid the biological and pathological foundation for the study of the urinary system in this plateau animal.

MATERIALS AND METHODS

Animals

Fifteen highland-plateau yaks were used for the study. Five of them were purchased in Gannan Tibetan Autonomous Prefecture, and the rest were purchased in Qinghai Province. This study was approved by the State Forestry Administration, and all procedures were performed in compliance with guidelines for the care and use of laboratory animals adopted by the Ministry of Science and Technology of the People's Republic of China.

Fifteen clinically normal yaks were divided into the following three age groups: newborn (1–7 days, n = 5), adult (3–6 years, n = 5) and old (7–10 years, n = 5). All groups included both males and females. Each yak was euthanized with pentobarbital sodium (200 mg/kg, IV). The kidney was harvested at 10–30 min after euthanasia. For histologic and immunohistochemical analyses, small specimens of kidney tissue were fixed in a solution of 4% paraformaldehyde in a phosphate buffer (pH, 7.3).

Light microscopy

Paraformaldehyde-fixed tissue specimens were embedded in paraffin. The tissue was then sliced into four μm-thick sections, and the distribution of collagen and basic structure in the kidney was observed by Masson's trichrome (aniline blue) staining,

and the structure of the arched artery was observed by AB-PAS/Masson's trichrome (aniline blue) double staining. Haematoxylin and eosin (H&E) staining was used to observe the difference in kidney structure at a different age. The above structures were observed by optical microscope (Olympus DP71, Tokyo).

Immunohistochemical analysis

For immunohistochemical staining to investigate EGFR, BMP-2 and p53 expression levels, kidney tissue sections were deparaffinised in xylene and rehydrated through a graded series of alcohols. The antigen was repaired with 3% deionised H2O2 (15–20 min) and sealed with goat serum (15–20 min). Primary rabbit anti-EGFR, anti-BMP-2 and anti-p53 polyclonal antibodies (Bioss, Beijing, 1:200 dilution) were applied and sections were incubated at 4°C overnight. The sections were then incubated with the goat anti-rabbit antibody and peroxidase complex. The labelled sections were then counterstained with 3-3'diaminobenzidine [10] and the nucleus lightly counterstained with haematoxylin.

Statistical analysis

Intensity measurements for immunohistochemical assay were performed using integrated optical density and measured by Image analysis software (Image-Pro Plus version 6.0 Media Cybernetics, USA). Statistical analysis was performed using the Statistical Package for Social Science software (version 19.0, IBM Corp, Armonk). Values of $p < 0.05$ were considered significant.

RESULTS

Histologic characteristics

The surface of the yak kidney was covered by a membrane that composed of collagen fibres (Fig. 1A) and under the membrane was the substance. Substance included cortex (Fig. 1C) and medulla (Fig. 1D) which were bounded by the arched artery (Fig. 1B). In the cortex, there were renal corpuscles, renal tubules and collecting tubules. The ellipsoidal renal corpuscles were respectively glomeruli and renal capsule from inside to outside. The glomeruli were mainly composed of a network of capillaries and the wall of the renal capsule was rich in collagen fibres (Fig. 1A). The bend part of renal tubules included proximal convoluted tubule and distal convoluted tubule. In the cortex, the collecting tubules were divided into two parts, which were bow type tubules and straight set tubules (Fig. 1C). The medulla included straight parts of renal tubules (proximal straight tubule and distal straight tubule); thin segments and collecting tubules in the depths of the medulla were generally referred to nipple tubes (Fig. 1D). In addition, the cortical and medullary tubules were rich in collagen fibres.

The number of glomerular in the kidney cortex of yak decreased with age. The size of glomeruli increased with age and the distribution of cells in the newborn yak glomeruli was closed, especially in the marginal region, where the cells were closely aligned. However, the gap between adult and elderly glomeruli cells became larger, the cells were loosely arranged, and the number of cells in the marginal zone decreased (Fig. 2A–C).

The study on the structure of renal tubules and collecting tubules in yak kidney of all age groups were shown in Figure 2A–F. The results showed that compared with the adult and old yak renal tubules, newborn yak renal tubular structure had not been fully developed, and distal tubule wall was not obvious, but the proximal tubule was obvious.

Immunohistochemical localisations

Distribution characteristics of EGFR in the kidney of the yak. The EGFR positive reaction was mainly distributed in the proximal tubule epithelial cells of the newborn group (Fig. 3A, D), but in the adult (Fig. 3B, E) and old group (Fig. 3C, F), the renal tubular epithelial cells were extensively distributed and the collecting tubule epithelial cells and glomerular cells demonstrated slight distribution.

Distribution characteristics of BMP-2 in the kidney of the yak. The positive reaction of BMP-2 in the newborn group mainly distributed in the proximal tubule epithelial cells (Fig. 4A, D). The positive reaction of BMP-2 in the adult group (Fig. 4B, E) and the old group (Fig. 4C, F) was widely distributed in the renal tubular epithelial cells and the collecting tubule epithelial cells. The macroscopic observation found that distal tubules positive reaction was more than the proximal tubule positive reaction.

Distribution characteristics of p53 in the kidney of the yak. p53 positive reactions were widely distributed in all age groups of glomerular cells, renal tubular epithelial cells and collecting tubule epithelial cells. The macroscopic observation found that the positive reaction was more intense in distal tubular epithelial cells (Fig. 5A–F).

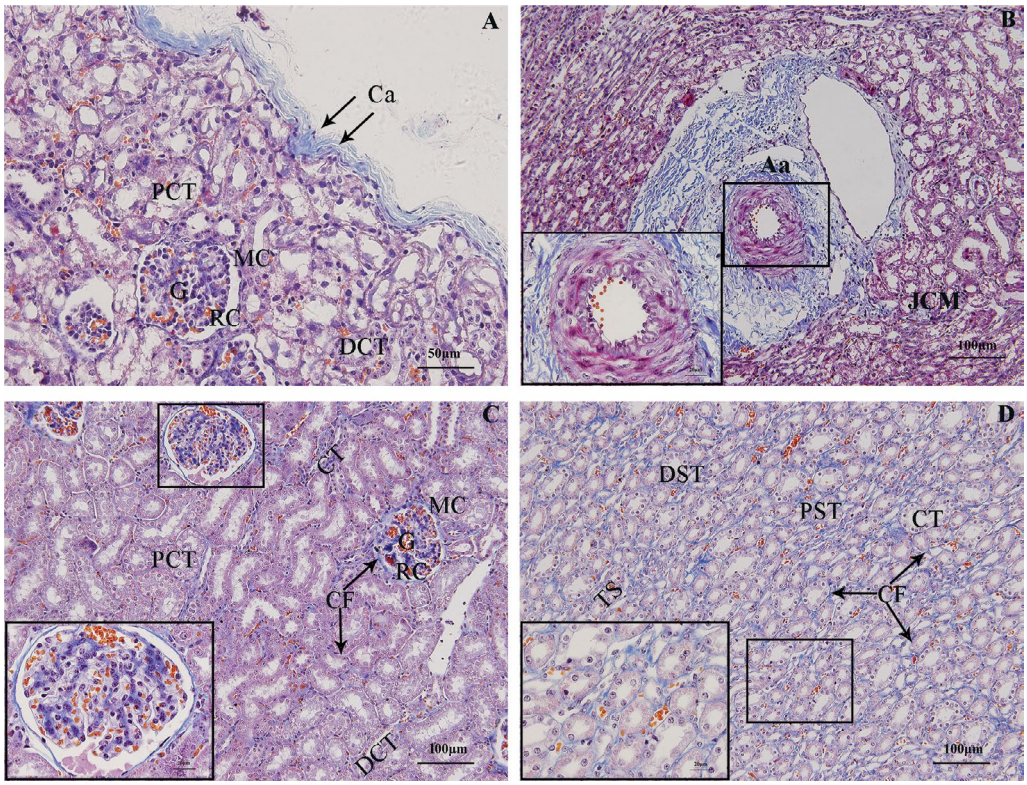

Figure 1. Representative photomicrographs of kidney sections from healthy highland-plateau yaks in two age groups (newborn [1 to 7 days old; **A** and **B**], old [7 to 10 years old; **C** and **D**]) illustrating histologic characteristics (A through D). To assess the histologic characteristics, sections were stained with Masson's trichrome (aniline blue) and AB-PAS/Masson's trichrome (aniline blue) double stain. Bar = 100 μm. Inserts in panels C through D: Higher-magnification views of the arched artery, renal capsule and renal tubules. Bar = 20 μm. Ca — capsule; MC — malpighian corpuscle; G — glomeruli; RC — renal capsule; PCT — proximal convoluted tubule; DCT — distal convoluted tubule; Aa — arched artery; JCM — junction of cortex and medulla; CT — collecting tubule; CF — collagen fibre; PST — proximal straight tubule; DST — distal straight tubule; TS — thin segment.

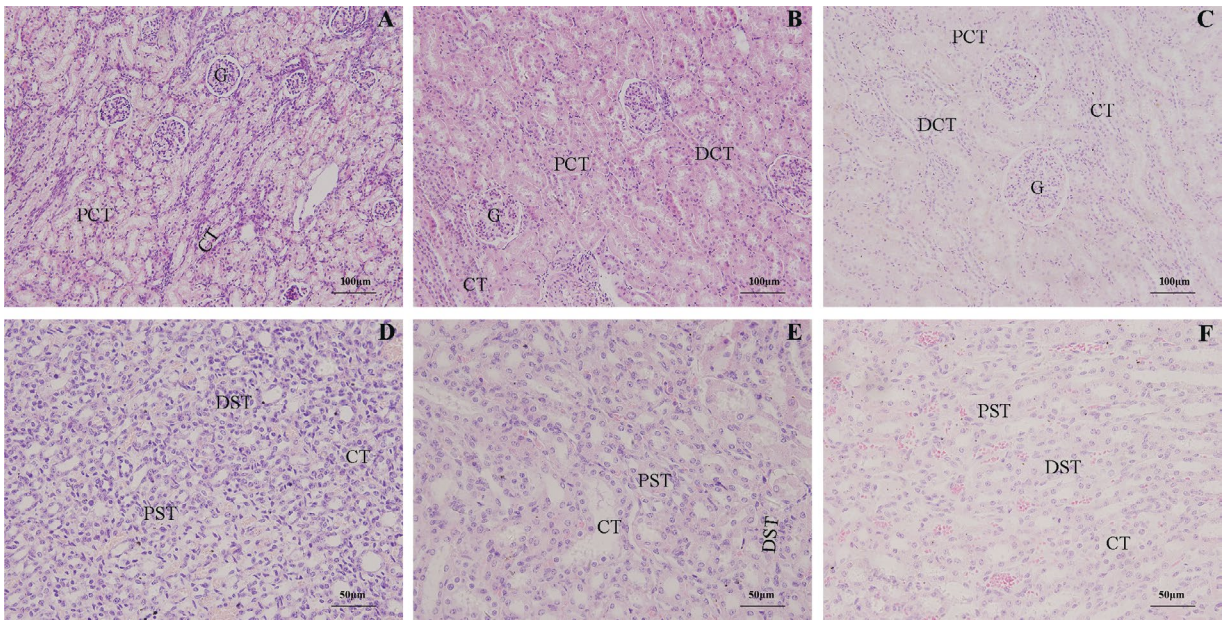

Figure 2. Representative photomicrographs of kidney sections from healthy highland-plateau yaks in three age groups (newborn [1 to 7 days old; **A** and **D**], adult [3 to 6 years old; **B** and **E**], or old [7 to 10 years old; **C** and **F**]) illustrating histologic changes (A through F). To assess the histologic changes, sections were stained with H&E stain. A through C: bar = 100 μm. D through F: bar = 50 μm; G — glomeruli; PCT — proximal convoluted tubule; DCT — distal convoluted tubule; CT — collecting tubule; PST — proximal straight tubule; DST — distal straight tubule.

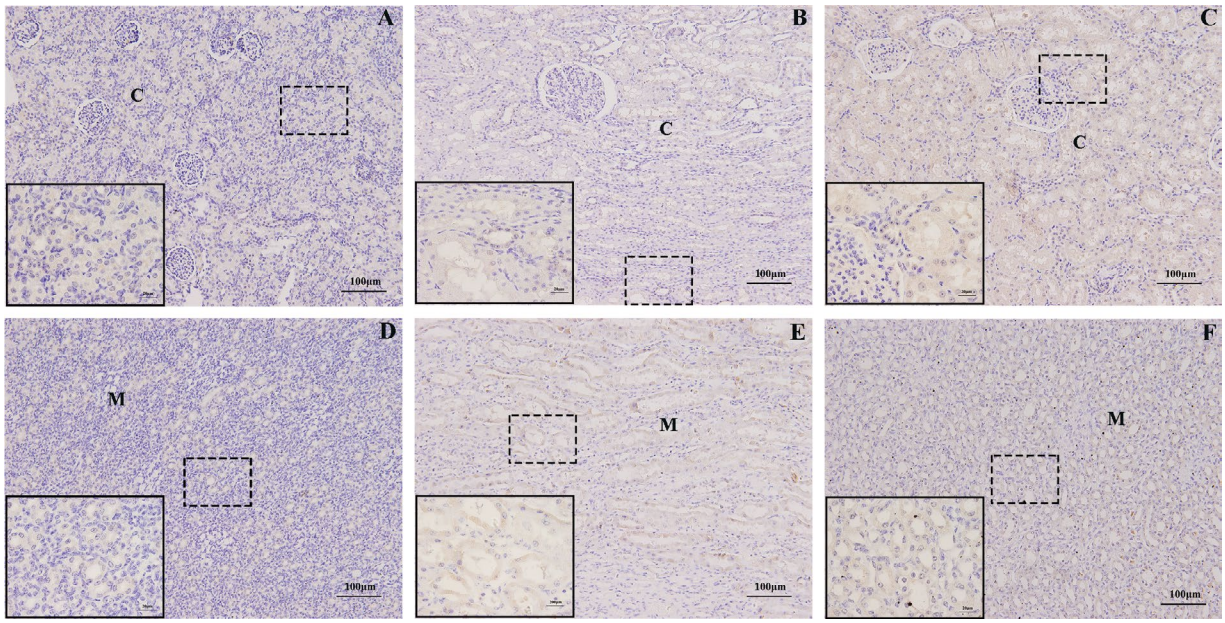

Figure 3. Representative photomicrographs of kidney sections from healthy highland-plateau yaks in three age groups (newborn [1 to 7 days old; **A** and **D**], adult [3 to 6 years old; **B** and **E**], or old [7 to 10 years old; **C** and **F**]) illustrating the localisation of epidermal growth factor receptor (EGFR) (A through F). Immunohistochemical staining was used to identify EGFR, which appears in yellow. Bar = 100 μm. Inserts in panels A through F: Higher-magnification views of the positive expression of EGFR in the kidney. Bar = 20 μm; C — cortex; M — medulla. See Figure 1 for the remainder of the key.

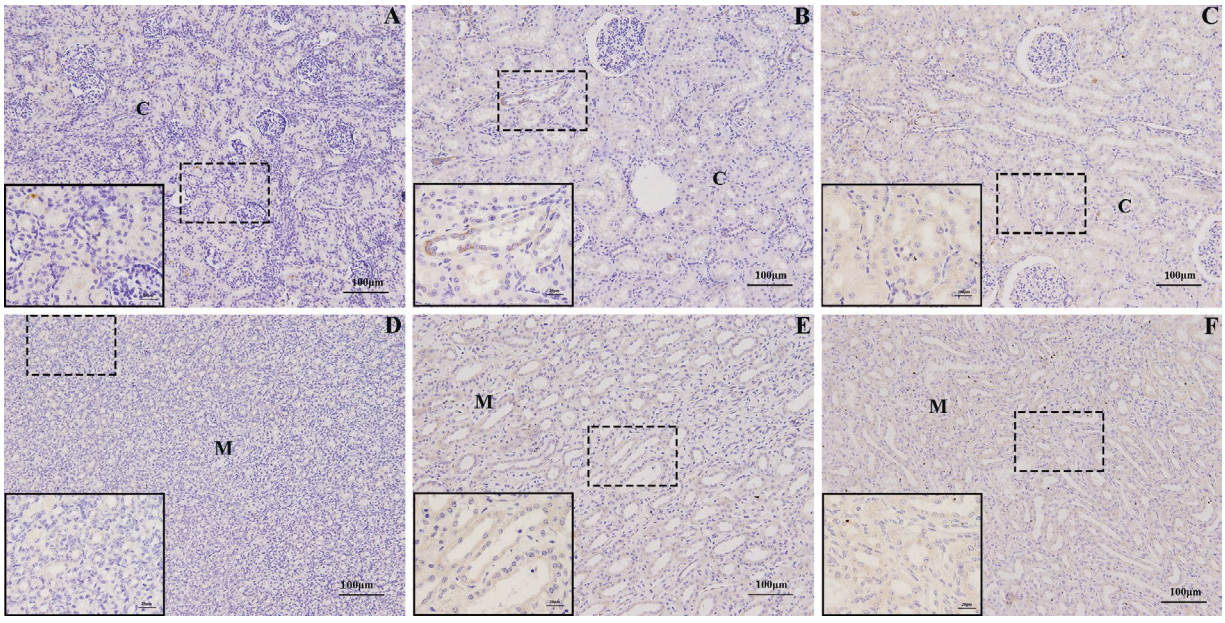

Figure 4. Representative photomicrographs of kidney sections from healthy highland-plateau yaks in three age groups (newborn [1 to 7 days old; **A** and **D**], adult [3 to 6 years old; **B** and **E**], or old [7 to 10 years old; **C** and **F**]) illustrating the localisation of bone morphogenetic protein-2 (BMP-2) (A through F). Immunohistochemical staining was used to identify BMP-2, which appears in yellow. Bar = 100 μm. Inserts in panels A through F: Higher-magnification views of the positive expression of BMP-2 in the kidney. Bar = 20 μm; C — cortex; M — medulla. See Figure 1 for the remainder of the key.

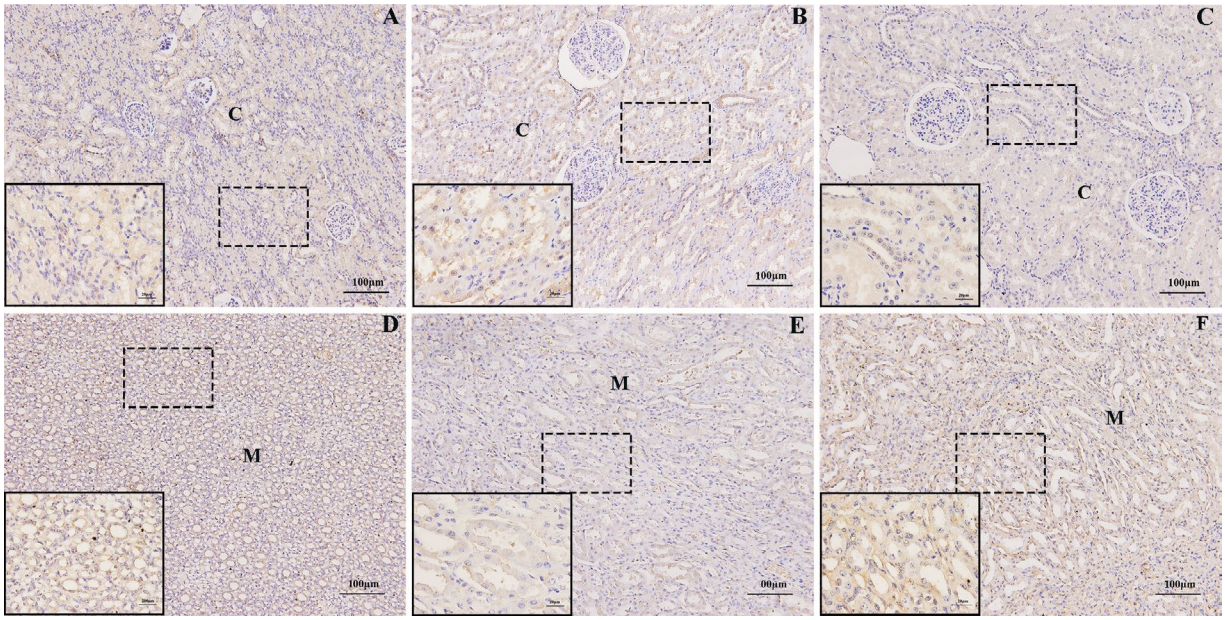

Figure 5. Representative photomicrographs of kidney sections from healthy highland-plateau yaks in three age groups (newborn [1 to 7 days old; **A** and **D**], adult [3 to 6 years old; **B** and **E**], or old [7 to 10 years old; **C** and **F**]) illustrating the localisation of p53 (A through F). Immunohistochemical staining was used to identify p53, which appears in yellow. Bar = 100 μm. Inserts in panels A through F. Higher-magnification views of the positive expression of p53 in the kidney. Bar = 20 μm; C — cortex; M — medulla. See Figures 1 and 2 for the remainder of the key.

Measurements of immunohistochemistry

Optical density values of EGFR in the kidney of the yak

Different regions. EGFR positive responses were different in different regions of the same age group. In the newborn group, the positive expression of EGFR in the medulla was significantly higher than the cortex and the junction of cortex and medulla (Fig. 6A, $p < 0.05$); In the adult group, the positive expression of EGFR in the junction of the cortex and medulla was lower than the cortex and medulla; In the old group, the positive expression of EGFR in the medulla of the old group was lower than the cortex and the junction of cortex and medulla. There was no significant difference between the adult and the old groups in three regions (Fig. 6A, $p > 0.05$).

Different age groups. With age, the positive expression of EGFR in the cortex and the junction of cortex and medulla of the newborn group was significantly lower than the adult group and the old group (Fig. 6B, $p < 0.05$), while the EGFR positive expression in medulla were the largest in aged, followed by the differences in the newborn, with those in the adult being the smallest and there were significant differences between the newborn and the adult group and the adult and old age groups (Fig. 6B, $p < 0.05$).

Optical density values of BMP-2 in the kidney of the yak

Different regions. The positive expression of BMP-2 in the cortex of the newborn and the old group was higher than the junction of cortex and medulla and the medulla, and there was no significant difference between the three regions (Fig. 7A, $p > 0.05$). The positive expression of BMP-2 in the medulla of the adult group was significantly lower than the cortex and the junction of cortex and medulla (Fig. 7A, $p < 0.05$).

Different age groups. The expression of BMP-2 positive expression in cortex, the junction of cortex and medulla and medulla were increased with age. The positive expression in the cortex and medulla of the old group was significantly different from the newborn group and the adult group (Fig. 7B, $p < 0.05$). However, there was no significant difference between the different age groups of the junction of cortex and medulla (Fig. 7B, $p > 0.05$).

Optical density values of p53 in the kidney of the yak

Different regions. The positive expression of p53 in the cortex of the newborn group was higher than the junction of cortex and medulla and the medulla. The positive expression of p53 in the medulla of the

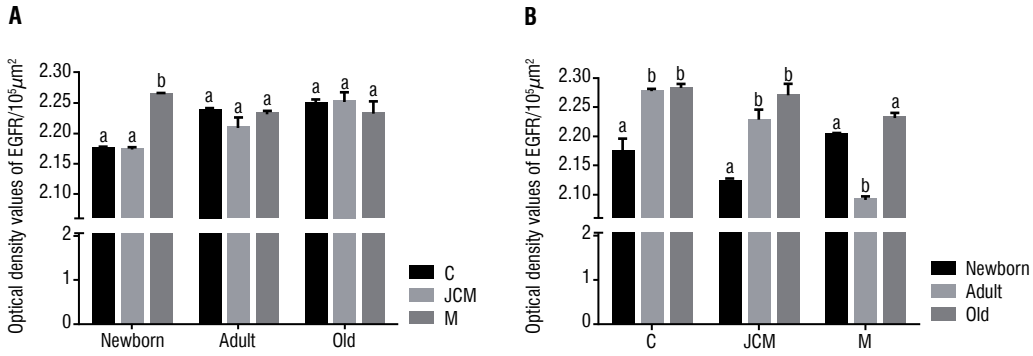

Figure 6. The optical density value distribution of epidermal growth factor receptor (EGFR) in the different regions of the kidney of same age groups of yaks (Fig. 4A). The optical density value distribution of EGFR in the same regions of the kidney of different age groups of yaks (Fig. 4B); C — cortex; JCM — junction of cortex and medulla; M — medulla. Data are reported as mean ± standard deviation. Mean values with different letters are significantly different ($p < 0.05$) and same letters are not significantly different ($p > 0.05$). [a]Not significantly; [b]Significantly different.

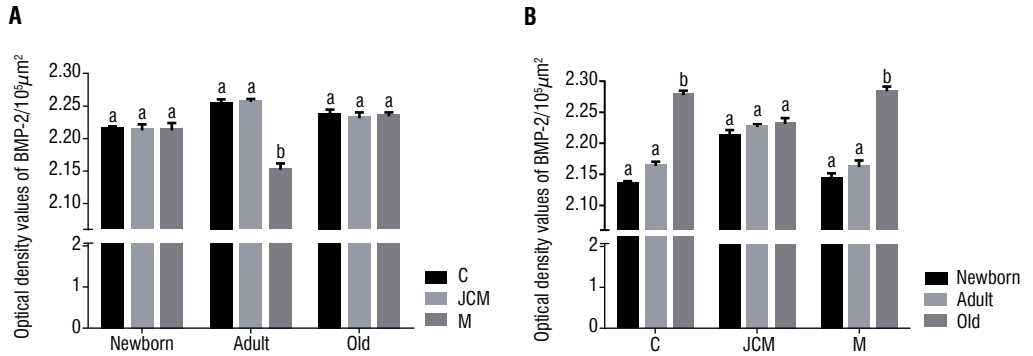

Figure 7. The optical density value distribution of bone morphogenetic protein-2 (BMP-2) in the different regions of the kidney of same age groups of yaks (Fig. 6A). The optical density value distribution of BMP-2 in the same regions of the kidney of different age groups of yaks (Fig. 6B); C — cortex; JCM — junction of cortex and medulla; M — medulla. Data are reported as mean ± standard deviation. Mean values with different letters are significantly different ($p < 0.05$) and same letters are not significantly different ($p > 0.05$). [a]Not significantly; [b]Significantly different.

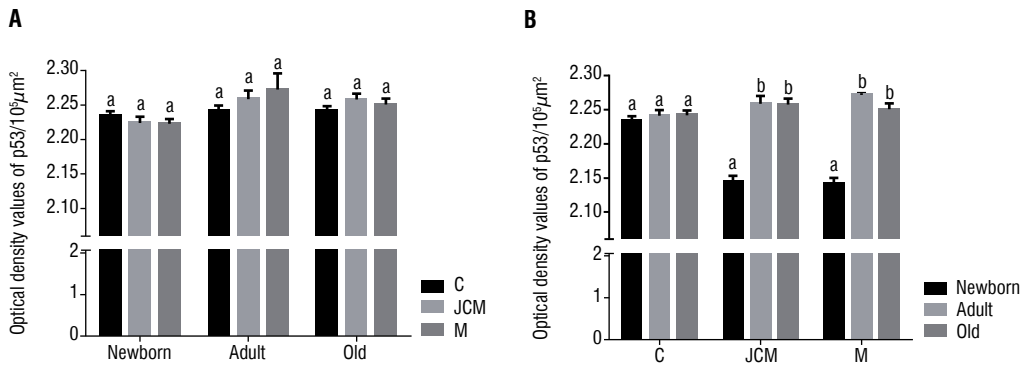

Figure 8. The optical density value distribution of p53 in the different regions of the kidney of same age groups of yaks (Fig. 8A). The optical density value distribution of p53 in the same regions of the kidney of different age groups of yaks (Fig. 8B); C — cortex; JCM — junction of cortex and medulla; M — medulla. Data are reported as mean ± standard deviation. Mean values with different letters are significantly different ($p < 0.05$) and same letters are not significantly different ($p > 0.05$). [a]Not significantly; [b]Significantly different.

adult group was higher than the cortex and the junction of cortex and medulla. The positive expression of p53 in the junction of cortex and medulla of the old group was higher than the cortex and medulla. Different regions among the three groups were not significant (Fig. 8A, $p > 0.05$).

Different age groups. The positive expression of p53 in the cortex of the old group was higher

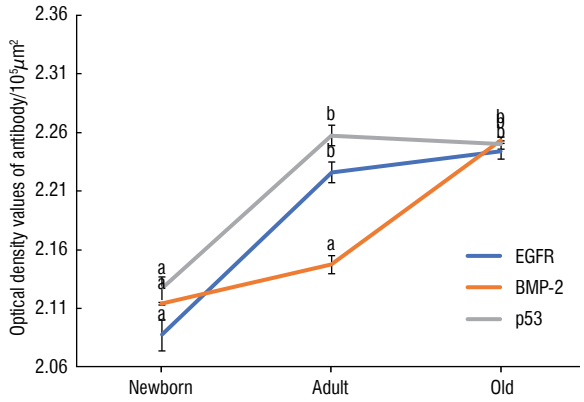

Figure 9. The optical density values of epidermal growth factor receptor (EGFR), bone morphogenetic protein-2 (BMP-2) and p53 in the kidney of different age groups of yaks. Data are reported as mean ± standard deviation. Mean values with different letters are significantly different ($p < 0.05$) and same letters are not significantly different ($p > 0.05$). [a]Not significantly; [b]Significantly different.

than the adult and the newborn, and there was no significant difference between the three age groups (Fig. 8B, $p > 0.05$). The positive expression of p53 in the junction of cortex and medulla and medulla of the newborn group was significantly lower than the adult and the old (Fig. 8B, $p < 0.05$).

Comparison of the optical density values of EGFR, BMP-2 and p53 in different age of the yak kidneys

The total positive expression of EGFR and BMP-2 increased with age, while the total positive expression of p53 first increased and then decreased. The total positive expression of EGFR and p53 in the newborn was significantly different from the adult and the old (Fig. 9, $p < 0.05$); the total positive expression of BMP-2 in the old was significantly different from the newborn and the adult (Fig. 9, $p < 0.05$).

DISCUSSION

In this study, the tissue structure of yak kidney was demonstrated by Masson's trichrome (aniline blue) stain and AB-PAS/Masson's trichrome (aniline blue) double stains. The results showed that the kidney of yaks was like human [28], mouse [24], rabbit [21], Bactrian camels [36] and African white rhinoceros [26]. This result provided a reference for the histological changes of clinically normal yak kidney. Histological characteristics of postnatal day 11 rat kidney show that the most glomeruli, particularly in the outer cortex are immature appearing. Tubules are not fully mature [32]. This study found that the kidney structure of newborn yak was not fully developed, which was consistent with the previous research. However, we also found that there was no intrinsic change in the internal tissue structure of yak kidney, but the development degree of the glomerular, renal tubule and the collection tubule had changed. This result is consistent with the studies of the human kidney [18, 34]. It showed that the kidney structure tended to be completed with age, and the function of the kidney gradually increased [7].

Epidermal growth factor receptor positive reactions were widely distributed in the kidneys, including mesangial cells, proximal tubule, and cortical and inner medullary collecting duct, as well as in medullary interstitial cells [11]. We found that the EGFR positive reaction was mainly distributed in the proximal tubule epithelium of the newborn yak and were widely distributed in the adult and old yak kidneys. Our results were not consistent with the above researchers. It indicated that the function of the nephron of the newborn yak was not fully developed and its function was not comprehensive. However, EGFR was present in the kidney before birth and was involved in cell proliferation and differentiation. In the newborn, the proximal tubules are developed and mature and the existence of EGFR is to maintain the integrity of the mature tubule epithelium [20]. BMP-2 was essential for early embryonic development [39]. In vertebrates, BMP genes were multi-effect growth/differentiation factors and expressed in many embryonic organs and tissues, including epithelial and mesenchymal cells [16]. Previous studies found that BMP-2 was highly expressed in the heart myocardial layer and uterine epithelial cells of the mice [23]. Up to now, there is no relevant literature on BMP-2 expression in the kidney. Our study found that the positive reaction of BMP-2 in the newborn group was mainly in the proximal tubule epithelium and that in the adult group and the old group they were widely distributed in the kidney. Therefore, we speculate that BMP-2 may have a role in promoting kidney development. Molchadsky et al. [29] found that p53 expression is ubiquitous early in mouse embryogenesis, after which time expression is restricted to specific tissues during organogenesis. The study found that p53 positive reaction was widely distributed in the newborn yak, suggesting that p53 was involved in cell migration, metabolism, differentiation, and self-renewal during this period [9, 31].

The results of the average optical density values of the kidney cortex, the junction of cortex and medulla and medulla of different age groups indicate that the positive expression of EGFR in the cortex and the junction of cortex and medulla increased with age, while the positive expression of EGFR in the medulla decreased first and then increased. The results indicate that EGFR promoted kidney development [30]. EGFR has existed from embryonic stage, and the tissues derived from endoderm and ectoderm can promote mitosis and stimulate the synthesis and metabolism, and stimulate the proliferation of epidermal cells, epithelial cells, interstitial cells and vascular endothelial cells. Therefore, there was a change in the degree of epithelial cell integrity with age, and there was an incremental change in the cortex and the junction of the cortex and medulla, and the EGFR content in the tubules of the medulla were reduced after birth. With age, EGF binds to the receptor to form a dimer, which activates the tyrosine kinase receptor in the receptor cell. The enzyme receptor itself or substrate phosphorylation on tyrosine residues, result in a series of downstream signal protein and enzyme phosphorylation (activation). By means of MAPK, the proliferation signal was introduced into the nucleus, which eventually caused the cell to enter the S stage, leading to cell proliferation and increasing the expression of EGFR [4]. In addition, some results suggested that BMP-2 could promote embryonic development. In this study, the positive expression of BMP-2 in the cortex, medulla and the junction of cortex and medulla increased with age. Therefore, it is speculated that BMP-2 has been involved in the proliferation and differentiation of cells in the kidney at the beginning of the embryonic period and promoted the development of the kidneys [17]. At the same time, the study showed that positive expression of p53 in cortex increased with age, while positive expression of p53 in the medulla and the junction of cortex and medulla first increased and then decreased with age. The results indicated that the development of the cortex, the junction of cortex and medulla and medulla in the kidney was different, and the p53 showed different positive expression changes with the development degree of each structure [29].

Finally, the results also demonstrated that the total positive expression of EGFR and BMP-2 increased with age and the total positive expression of p53 decreased with age. Previous literature confirms that EGFR and BMP-2 are expressed in organs such as kidneys [6] and uterus [23] and increased expression with age. Therefore, we hypothesize that EGFR and BMP-2 have the effect of promoting kidney development. He et al. [12] found that p53 protein expression levels decreased in the kidneys of rats with age, which was consistent with our results, indicating that p53 was a key regulatory factor in the development of kidney.

CONCLUSIONS

The results revealed that the development of the kidney of the yak internal structure changed with age, indicating that the yak kidney structure tended to complete with age and the function of kidneys gradually improved. EGFR and BMP-2 positive reaction in the newborn group was mainly distributed in the proximal tubule epithelial cells, and widely distributed in the adult and old groups, indicating that EGFR and BMP-2 had the effect of promoting kidney development. However, p53 had been widely distributed in newborn kidney of the yaks. It is suggested that p53 had been involved in cell migration and metabolic differentiation and self-renewal in the new stage.

Acknowledgements

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REFERENCES

1. Attisano L, Wrana JL. Mads and Smads in TGF beta signalling. Curr Opin Cell Biol. 1998; 10(2): 188–194, indexed in Pubmed: 9561843.
2. Bauchet AL, Masson R, Guffroy M, et al. Immunohistochemical identification of kidney nephron segments in the dog, rat, mouse, and cynomolgus monkey. Toxicol Pathol. 2011; 39(7): 1115–1128, doi: 10.1177/0192623311425060, indexed in Pubmed: 22006284.
3. Baserga M, Hale MA, Ke X, et al. Uteroplacental insufficiency increases p53 phosphorylation without triggering the p53-MDM2 functional circuit response in the IUGR rat kidney. Am J Physiol Regul Integr Comp Physiol. 2006; 291(2): R412–R418, doi: 10.1152/ajpregu.00880.2005, indexed in Pubmed: 16914427.
4. Ciardiello F, Tortora G. EGFR antagonists in cancer treatment. N Engl J Med. 2008; 358(11): 1160–1174, doi: 10.1056/NEJMra0707704, indexed in Pubmed: 18337605.
5. El-Dahr S, Hilliard S, Aboudehen K, et al. The MDM2-p53 pathway: multiple roles in kidney development. Pediatr Nephrol. 2014; 29(4): 621–627, doi:10.1007/s00467-013-2629-y, indexed in Pubmed: 24077661.
6. Gattone VH, Sherman DA, Hinton DA, et al. Epidermal growth factor in the neonatal mouse salivary gland and kidney. Biol Neonate. 1992; 61(1): 54–67, doi: 10.1159/000243531, indexed in Pubmed: 1567929.
7. Gekle M. Kidney and aging: a narrative review. Exp Gerontol. 2017; 87(Pt B): 153–155, doi: 10.1016/j.exger.2016.03.013, indexed in Pubmed:27032877.

8. Gesualdo L, Di Paolo S, Calabró A, et al. Expression of epidermal growth factor and its receptor in normal and diseased human kidney: an immunohistochemical and in situ hybridization study. Kidney Int. 1996; 49(3): 656–665, indexed in Pubmed: 8648906.
9. Gottlieb E, Vousden KH. p53 regulation of metabolic pathways. Cold Spring Harb Perspect Biol. 2010; 2(4): a001040, doi:10.1101/cshperspect.a001040, indexed in Pubmed: 20452943.
10. Gu W, Yang L, Wang S, et al. Generation and application of a novel InsP(3)R(1) mono-antibody from mouse. J Immunoassay Immunochem. 2015; 36(5): 487–495, doi: 10.1080/15321819.2014.996817, indexed in Pubmed: 25522905.
11. Harris RC. Potential physiologic roles for epidermal growth factor in the kidney. Am J Kidney Dis. 1991; 17(6): 627–630, indexed in Pubmed: 2042635.
12. He X, Xie Z, Dong Q, et al. Dynamic p53 protein expression and phosphorylation in the kidneys of rats that experienced intrauterine growth restriction. Ren Fail. 2015; 37(5): 896–902, doi: 10.3109/0886022X.2015.1015428, indexed in Pubmed: 25721428.
13. He Y, Yu S, Hu J, et al. Changes in the Anatomic and Microscopic Structure and the Expression of HIF-1α and VEGF of the Yak Heart with Aging and Hypoxia. PLoS One. 2016; 11(2): e0149947, doi: 10.1371/journal.pone.0149947, indexed in Pubmed: 26914488.
14. Heldin CH, Miyazono K, ten Dijke P. TGF-beta signalling from cell membrane to nucleus through SMAD proteins. Nature. 1997; 390(6659): 465–471, doi: 10.1038/37284, indexed in Pubmed: 9393997.
15. Hilliard S, Aboudehen K, Yao X, et al. Tight regulation of p53 activity by Mdm2 is required for ureteric bud growth and branching. Dev Biol. 2011; 353(2): 354–366, doi: 10.1016/j.ydbio.2011.03.017, indexed in Pubmed: 21420949.
16. Hogan BL. Bone morphogenetic proteins: multifunctional regulators of vertebrate development. Genes Dev. 1996; 10(13): 1580–1594, indexed in Pubmed: 8682290.
17. Hogan BL. Bone morphogenetic proteins in development. Curr Opin Genet Dev. 1996; 6(4): 432–438, indexed in Pubmed: 8791534.
18. Hommos MS, Glassock RJ, Rule AD. Structural and functional changes in human kidneys with healthy aging. J Am Soc Nephrol. 2017; 28(10): 2838–2844, doi: 10.1681/ASN.2017040421, indexed in Pubmed: 28790143.
19. Iwasaki S, Tsuruoka N, Hattori A, et al. Distribution and characterization of specific cellular binding proteins for bone morphogenetic protein-2. J Biol Chem. 1995; 270(10): 5476–5482, indexed in Pubmed: 7890664.
20. Jung JY, Song JH, Li C, et al. Expression of epidermal growth factor in the developing rat kidney. Am J Physiol Renal Physiol. 2005; 288(1): F227–F235, doi: 10.1152/ajprenal.00058.2004, indexed in Pubmed: 15353402.
21. Kozma C. Anatomy, Physiology, and Biochemistry of the Rabbit. The biology of the laboratory rabbit. 1974; 12(1): 55–58, doi: 10.1016/c2013-0-11681-9, indexed in Pubmed: 94355043.
22. Kretzschmar M, Doody J, Massagué J. Opposing BMP and EGF signalling pathways converge on the TGF-beta family mediator Smad1. Nature. 1997; 389(6651): 618–622, doi: 10.1038/39348, indexed in Pubmed: 9335504.
23. Li Y, Wei Qw, Feng Jg, et al. Expression of bone morphogenetic protein 2, 4, and related components of the BMP signaling pathway in the mouse uterus during the estrous cycle. J Zhejiang Univ Sci B. 2014; 15(7): 601–610, doi: 10.1631/jzus.B1300288, indexed in Pubmed: 25001220.
24. Liebelt A. Unique Features of Anatomy, Histology, and Ultrastructure Kidney, Mouse. Urinary System. Springer Berlin Heidelberg. 1998: 37–57.
25. Liu P, Yu S, Cui Y, et al. Cloning of HSP90, expression and localization of HSP70/90 in different tissues including lactating/non-lactating yak (Bos grunniens) breast tissue. PLoS One. 2017; 12(7): e0179321, doi: 10.1371/journal.pone.0179321, indexed in Pubmed: 28715410.
26. Lu ZQ, Peng KM, Zhang JB, et al. Histological Study on the African White Rhinoceros Kidney. Proceedings of the 17th Academic Symposium on Animal Anatomy and Tissue Embryology Branch of Chinese Association of Animal Sience and Veterinary Medicine. 2010: 285–288.
27. Massagué J. TGFbeta signaling: receptors, transducers, and Mad proteins. Cell. 1996; 85(7): 947–950, indexed in Pubmed: 8674122.
28. McBride J. Embryology, anatomy, and histology of the kidney. The Kidney. 2016: 1–18, doi: 10.1007/978-1-4939-3286-3_1.
29. Molchadsky A, Rivlin N, Brosh R, et al. p53 is balancing development, differentiation and de-differentiation to assure cancer prevention. Carcinogenesis. 2010; 31(9): 1501–1508, doi: 10.1093/carcin/bgq101, indexed in Pubmed: 20504879.
30. Pastore S, Mascia F, Mariani V, et al. The epidermal growth factor receptor system in skin repair and inflammation. J Invest Dermatol. 2008; 128(6): 1365–1374, doi: 10.1038/sj.jid.5701184, indexed in Pubmed: 18049451.
31. Schoppy DW, Ruzankina Y, Brown EJ. Removing all obstacles: a critical role for p53 in promoting tissue renewal. Cell Cycle. 2010; 9(7): 1313–1319, doi:10.4161/cc.9.7.11194, indexed in Pubmed: 20234190.
32. Seely JC. A brief review of kidney development, maturation, developmental abnormalities, and drug toxicity: juvenile animal relevancy. J Toxicol Pathol. 2017; 30(2): 125–133, doi: 10.1293/tox.2017-0006, indexed in Pubmed: 28458450.
33. Tang J, Liu Na, Zhuang S. Role of epidermal growth factor receptor in acute and chronic kidney injury. Kidney Int. 2013; 83(5): 804–810, doi:10.1038/ki.2012.435, indexed in Pubmed: 23325080.
34. Tauchi H, Tsuboi K, Okutomi J. Age changes in the human kidney of the different races. Gerontologia. 1971; 17(2): 87–97, indexed in Pubmed:5093734.
35. Tumelty KE, Higginson-Scott N, Fan X, et al. Identification of direct negative cross-talk between the SLIT2 and bone morphogenetic protein-Gremlin signaling pathways. J Biol Chem. 2018; 293(9): 3039–3055, doi: 10.1074/jbc.M117.804021, indexed in Pubmed: 29317497.
36. Wang WH, Chen HT. Studies on Comparative Histology of the Kidneys in Bactrian Camels (Camelus bactrianus). Journal of Lanzhou University. 2000; 36(4): 73–78, doi: 10.3321/j.issn:0455-2059.2000.04.006.
37. Xi Y, Shao F, Bai XY, et al. Changes in the expression of the Toll-like receptor system in the aging rat kidneys. PLoS One. 2014; 9(5): e96351, doi:10.1371/journal.pone.0096351, indexed in Pubmed: 24810370.
38. Yang B, Yu S, Cui Y, et al. Morphological analysis of the lung of neonatal yak. Anat Histol Embryol. 2010; 39(2): 138–151, doi: 10.1111/j.1439-0264.2009.00988.x, indexed in Pubmed: 20070291.
39. Zhang H, Bradley A. Mice deficient for BMP2 are nonviable and have defects in amnion/chorion and cardiac development. Development. 1996; 122(10): 2977–2986, indexed in Pubmed: 8898212.
40. Zhang Q, Yang K, Huang Y, et al. Distribution of T-cell markers CD4 and CD8α in lymphoid organs of healthy newborn, juvenile, and adult highland-plateau yaks. Am J Vet Res. 2017; 78(5): 609–617, doi: 10.2460/ajvr.78.5.609, indexed in Pubmed: 28441047.