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# Temporal and spatial expression of *Smads* and their correlation with *YAP1* expression in sheep

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**ABSTRACT.** The temporal and spatial patterns of *Smad* and Yes-associated protein 1 (*YAP1*) expression were investigated in skeletal muscle (gastrocnemius muscle and extensor digitorum longus) at different growth stages (2 days old, 2 and 6 months old) in Hu sheep. *Smads* were differentially expressed in sheep skeletal muscle, with high expression in the gastrocnemius muscle and lower expression in the extensor digitorum longus. Expression of *Smad2*, *Smad3*, and *Smad4* at the 2-day-old stage was significantly higher than at other stages ($P < 0.05$). The expression of *Smad7* in 2-day-old sheep was lower than in 6-month-old sheep, with the lowest levels at 2 months. *Smad* expression was higher in males than in females at the 2-day-old stage, and expression in 2- and 6-month-old males was lower than that in 2-day-old females. *Smad3* expression was higher in the 2-day- and 2-month-old males than in the females. There was a positive correlation (P < 0.01) between *YAP1* and *Smad2* expression in gastrocnemius muscle at the 2-month-old stage. *YAP1* and *Smad4*/*7* expression were positively correlated (P < 0.01) in extensor digitorum longus at the 2-day-old stage. *YAP1* expression was negatively correlated with *Smad7* in the extensor digitorum longus at 6 months. A significant difference between *Smad2* and *Smad3* (P < 0.01) expression in muscle was observed, consistent with *Smad3* and *Smad4* expression, indicating that these inhibit transforming growth factor-β signaling in the same way. There was a positive correlation (P < 0.01) between *YAP1* and *MSTN* expression, suggesting that *YAP1* participates in muscle growth in sheep.

**Key words:** Hu sheep; *Smads*; *YAP1*; *MSTN*; Gene expression

## INTRODUCTION

Yes-associated protein 1 (YAP1) is the major downstream effector of the Hippo signaling pathway and acts as a co-activator or a co-repressor. *YAP1* contains multiple domains and is involved in many intracellular signaling pathways. *YAP1* has important roles in cell proliferation and apoptosis, control of organ size, and tumorigenesis (Zhao et al., 2008; Li et al., 2011; Liu et al., 2011; Zhang and Zhu, 2011). The mechanism of action of gene products involved in the Hippo-*YAP* signaling pathway has gradually been clarified. The Hippo signaling pathway has been shown to interact with the transforming growth factor-β (TGF-β) signaling pathway and to co-regulate the cellular biology of organisms (Figure 1).

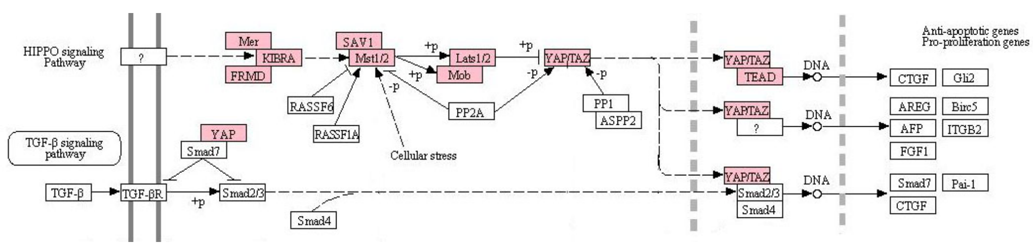


**Figure 1.** Signal integration in the transforming growth factor (TGF)-β and Hippo pathways.

TGF-β can both suppress growth and stimulate cellular differentiation through the intracellular messenger of mothers against decapentaplegic homolog (Smads) proteins. YAP1 acts as a transcriptional activator of Smads, which in turn, affect TGF-β signaling pathways (Fujii et al., 2012). The Smads are classified into three subgroups (Kaivo-oja et al., 2006): receptor smads (R-Smads), consisting by Smad2 and Smad3, common-mediator (Co-Smads), consisting of Smad4, and inhibitor smads (I-Smads), consisting of Smad6 and Smad7. The Smad signal transduction pathway involves extracellular ligands, cell surface receptors, and Smads, which initiate a signaling cascade that transmits the extracellular signal to the nucleus, thus regulating the transcription of target genes. Ligands in the Smad signal transduction pathway mainly belong to the TGF-β superfamily. YAP1 can bind Smad7, which has an antagonistic effect on TGF-β activity. Ferrigno et al. (2002) found that YAP1 could activate TGF-β receptor I (TβRI) to promote the expression of Smad7, and could also increase the ability of inhibiting activity of Smad7 to TGF-β signal transduction pathway. Watt et al. (2010) found that hYAP1 S127A overexpression could restrain myotube differentiation and that YAP1 S127 phosphorylation was necessary for the differentiation of myoblasts to myotubes.

Activation of the TGF-β pathway is important for muscle development, but few studies have investigated the effects of YAP1 on muscle growth and development. Because Smad7 is involved in the TGF-β signaling pathway, it is likely to interact with YAP1. Therefore, we hypothesized that YAP1 plays a significant role in muscle growth and development in Hu sheep by binding to Smad proteins through their WW domain, and through the subsequent inhibition or promotion of TGF-β activity.

## MATERIAL AND METHODS

### Materials used for expression

Hu sheep were used at three stages of development: 2-day-old, 2-month-old, and 6-month-old animals. For each stage, three male and three female lambs were used. For each stage, the gastrocnemius muscle and the extensor digitorum longus were collected. The present study was performed in accordance with the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 85-23, revised 1996) and was approved by the Animal Care and Use Committee of the Yangzhou University.

### Reagents and kits

Primer Script RT reagent kit and SYBR® Premix Ex Taq™ II were purchased from TaKaRa. Primers were designed by Oligo 7.0. Goldview was purchased from SBS Genetech Company, and all other reagents were purchased from China National Medicines Corporation Ltd.

### *Smad* gene expression

Total RNA was extracted with buffer containing β-mercaptoethanol and guanidine using an RNAiso plus kit (Takara Biotechnology Dalian, Co. Ltd., China). A total of 250 ng RNA was transcribed into cDNA from each sample using a Takara reverse transcription kit (Takara Biotechnology). Primers listed in Table 1 were used for q-PCR analysis.

**Table 1.** q-PCR primer sequences specific for *Smad*, *YAP1*, and 18S rRNA genes.

| Genes | Reference sequences | Primer sequences (5'-3') | Products (bp) |
|---|---|---|---|
| Smad2 | XM004020532 | SF: CTTGAGAAAGCCATCACCAC<br>SR: TCGATGGGACACCTGAAG | 180 |
| Smad3 | XM004010875 | SF: ATTGAGCTGCACCTGAACGGAC<br>SR: CTCCCTCTTCGCTCGCAGTGT | 116 |
| Smad4 | GAAI01002997 | SF: GAATAGCCCCAGCCATCAGT<br>SR: GCAACACAGCCTCTTGACTTCCG | 97 |
| Smad7 | GAAI01006724 | SF: CCCTCCAACTACTCGCTCCC<br>SR: GCAACACAGCCTCTTGACTTCCG | 90 |
| YAP1 | JQ714252 | SF: GACAGCGGACTGAGCATGAG<br>SR: CAGGGTGCTTTGGTTGATAGTG | 108 |
| 18S | AY753190 | SF: CGGCTACCACATCCAAGGAA<br>SR: GCTGGAATTACCGCGGCT | 187 |

## Data processing and statistical analysis

SPSS 16.0 was used to calculate the Ct values and standard errors of repeated samples, and the difference in relative gene expression was analyzed using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). The following equation was used to compare differences in gene expression in equivalent skeletal muscle types from Hu sheep of the same age and different sex: ΔΔCt was ΔCt (male) - ΔCt (female). The ΔΔCt was ΔCt (other months old) - ΔCt (2-day-old stage) when the sheep were the same sex, from the same skeletal muscle but were different ages. The ΔΔCt was ΔCt (extensor digitorum longus) - ΔCt (gastrocnemius muscle) when sheep were the same sex, age but samples were taken from different skeletal muscles. The value of sheep, with the same age, same skeletal muscle but different sex, was compared by *t*-test, while data on individuals of the same sex, using the same skeletal muscle but of different ages, and on individuals of the same sex, age but using samples from different skeletal muscles, were compared by one-way analysis of variance (ANOVA). In this analysis, the gastrocnemius muscle of 2-day-old female lambs served as the reference.

# RESULTS

## Spatial and temporal expression of *Smads* in sheep muscle

Expression of *Smad2* was higher in the gastrocnemius muscle than in the extensor digitorum longus. There were no significant differences in the expression of *Smad2* between sexes at the 2-month-old or 6-month-old stages, but in 2-day-old animals, there were significant differences in the expression of *Smad2* between the gastrocnemius muscle and the extensor digitorum longus in females (Figure 2I). The expression of *Smad3* was lower in the extensor digitorum longus than in the gastrocnemius muscle. There were no significant differences in the expression of *Smad3* between sexes at the 2-day- or 2-month-old stages, whereas in 6-month-old animals there were significant differences in expression between the gastrocnemius muscle and the extensor digitorum longus in females (Figure 2II). Expression of *Smad4* was lower in the extensor digitorum longus than in the gastrocnemius muscle. In 2-day- and 6-month-old sheep, there was no difference in the expression of *Smad4* between male and female animals, and in 2-month-old sheep, there was a significant difference in expression between the extensor digitorum longus and the gastrocnemius muscle in female animals (Figure 2III). Expression of *Smad7* in the extensor digitorum longus was lower than that in the gastrocnemius muscle. In 2-month-old animals, the expression of *Smad7* significantly differed between sexes, and in 2-day-old and 6-month-old animals, there were significant differences between expression in the extensor digitorum longus and gastrocnemius muscle in female sheep (Figure 2IV).

With increasing age, the expression of *Smad2* first decreased and then increased, with no significant difference observed between male and female sheep (Figure 3I). There was a highly significant difference in the expression of *Smad3* between male and female sheep at 2 days and 2 months of age (Figure 3II). The expression of *Smad4* first decreased and then increased in the gastrocnemius muscle of female lambs (Figure 3III). The expression of *Smad7* first decreased and then increased, with this expression being higher in 6-month-old animals than in 2-month-old animals. Significant or extremely significant differences exist between male and female lambs (Figure 3IV).

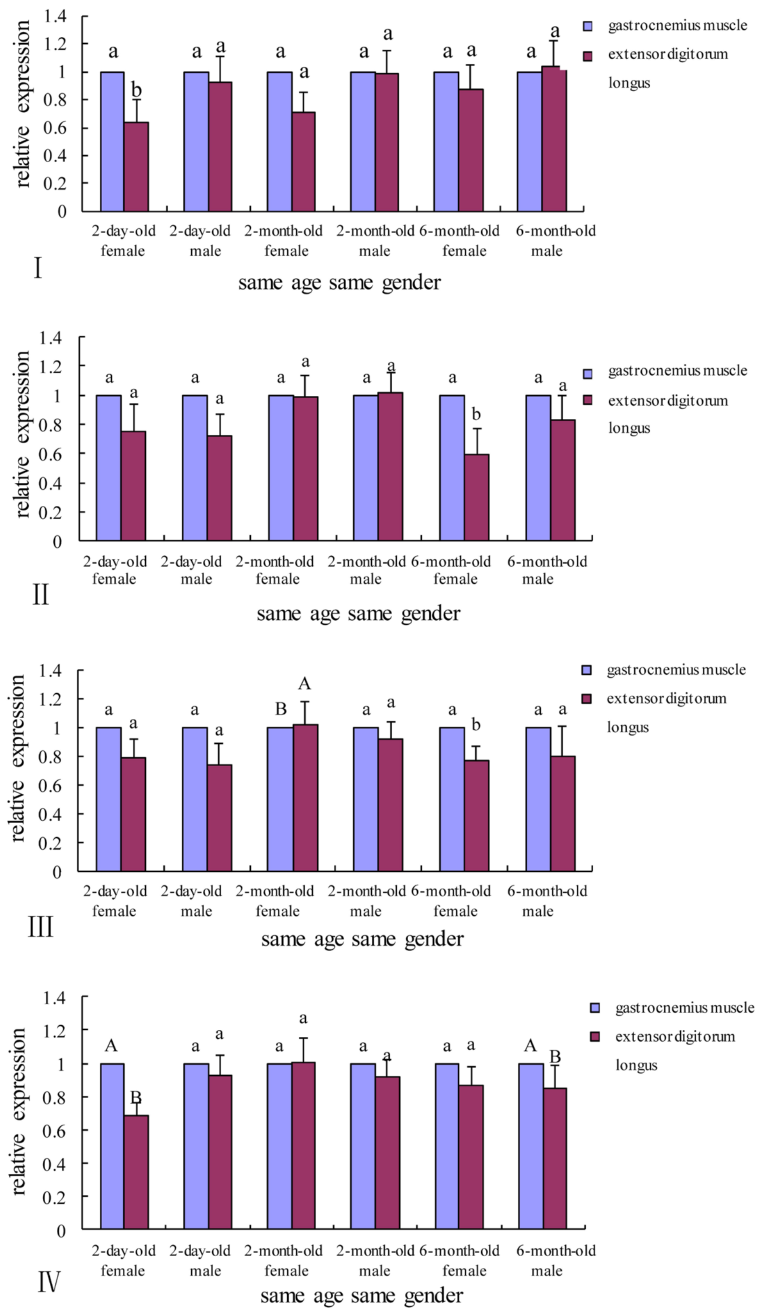


**Figure 2.** Expression of *Smads* in different muscles of sheep. **I.** Smad2; **II.** Smad3; **III.** Smad4; **IV.** Smad7. A, B, C, a, b, c indicate the results of multiple comparisons between the same sex and same growth stages in different muscles. The same letters denote no significant difference ($P > 0.05$). Values with different letters are significantly different ($0.01 < P < 0.05$). Values with different capitals are highly significantly different ($P < 0.01$). Different letters and capitals denote a significant difference ($0.01 < P < 0.05$) and a highly significant difference ($P < 0.01$).

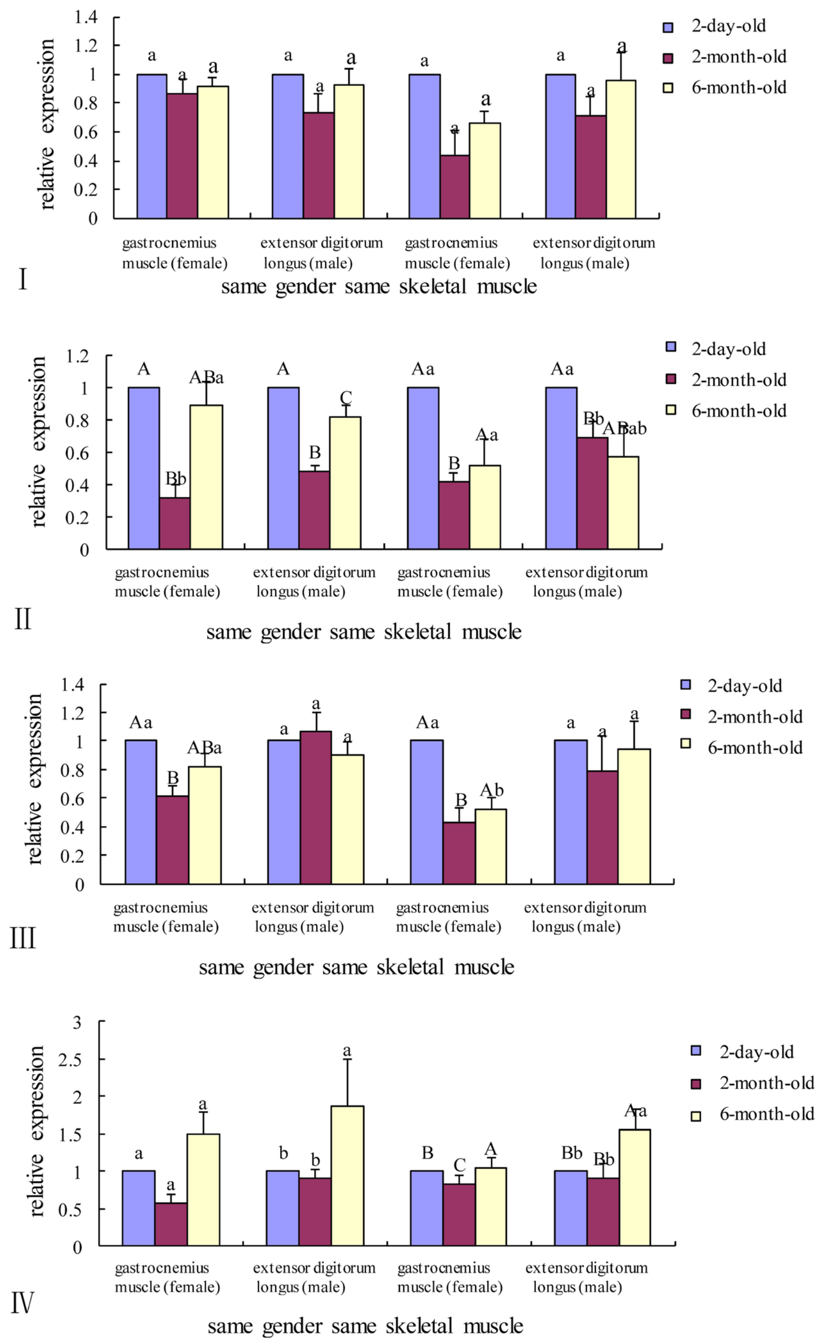


**Figure 3.** Expression of *Smads* in different growth stages of sheep. **I.** Smad2; **II.** Smad3; **III.** Smad4; **IV.** Smad7. A, B, C, a, b, c denote multiple comparisons between the same sex and same growth stages in different muscles. The same letters denote no significant difference (P > 0.05). Values with different letters are significantly different (0.01 < P < 0.05). Values with different capitals are highly significantly different (P < 0.01).

There was a significant difference in the expression of *Smad2* in gastrocnemius muscle between male and female lambs at 2 days of age. The expression of *Smad2* differed significantly in the gastrocnemius muscle of 2-month- and 6-month-old male and female lambs (Figure 4I). There was a significant difference in the expression of *Smad3* in the gastrocnemius muscle of lambs between sexes at 2 days of age. There was a highly significant difference in expression in the gastrocnemius muscle of lambs at 6 months of age between sexes (Figure 4II). There was a significant difference in the expression of *Smad4* in the extensor digitorum longus and gastrocnemius muscle at 2 days of age between male and female lambs. No significant difference was observed at 6 months of age between males and females (Figure 4III). A highly significant difference was observed in the expression of *Smad7* in the extensor digitorum longus between male and female lambs, and no difference was observed at other time points between sexes (Figure 4IV).

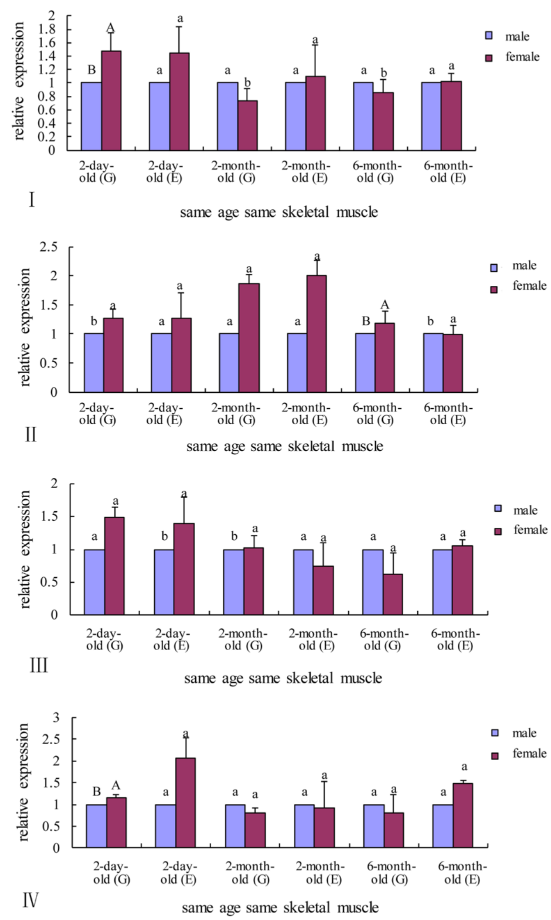


**Figure 4.** Expression of *Smads* in sheep of different genders. **I.** Smad2; **II.** Smad3; **III.** Smad4; **IV.** Smad7. A, B, C, a, b, c show the results of multiple comparisons between animals of the same sex and same growth stages in different muscles (G = gastrocnemius; E = extensor digitorum long). The same letters denote no significant difference ($P > 0.05$). Values with different letters are significantly different ($0.01 < P < 0.05$). Values with different capitals are highly significant different ($P < 0.01$).

## Association of *Smads* with *YAP1* gene expression

The expression of *Smad2* was positively associated with *Smad3*, *Smad4*, and *Smad7* expression in the gastrocnemius muscle (P < 0.01) in 2-day-old animals. The expression of *Smad3* was positively associated with *Smad4* and *Smad7* expression in the gastrocnemius muscle (P < 0.01). The expression of *Smad4* was highly significant (P < 0.01) and positively associated with *Smad7* in the gastrocnemius muscle (Table 2).

**Table 2.** Correlation between *Smads* and *YAP1* gene expression in the gastrocnemius muscle of 2-day-old animals.

| Groups | Smad2 | Smad3 | Smad4 | Smad7 | YAP1 | MSTN |
|---|---|---|---|---|---|---|
| Smad2 | 1 | 0.713** | 0.797** | 0.805** | 0.313 | 0.413 |
| Smad3 | 0.713** | 1 | 0.897** | 0.883** | 0.172 | 0.217 |
| Smad4 | 0.797** | 0.897** | 1 | 0.834** | 0.448 | 0.350 |
| Smad7 | 0.805** | 0.883** | 0.834** | 1 | 0.073 | 0.059 |
| YAP1 | 0.313 | 0.172 | 0.448 | 0.073 | 1 | 0.657** |
| MSTN | 0.413 | 0.217 | 0.350 | 0.059 | 0.657** | 1 |

In 2-month-old animals, the expression of *Smad2* was significantly and positively associated with *Smad3*, *Smad7*, and *YAP1* in the gastrocnemius muscle (P < 0.05). The expression of *Smad3* was highly significantly (P < 0.01) positively associated with *Smad7* in the gastrocnemius muscle (Table 3).

**Table 3.** Correlation between the expression of *Smads* and *YAP1* in the gastrocnemius muscle of 2-month-old animals.

| Groups | Smad2 | Smad3 | Smad4 | Smad7 | YAP1 | MSTN |
|---|---|---|---|---|---|---|
| Smad2 | 1 | 0.494* | 0.132 | 0.494* | 0.497* | 0.096 |
| Smad3 | 0.494* | 1 | 0.414 | 1.000** | -264 | -0.459 |
| Smad4 | 0.132 | 0.414 | 1 | 0.414 | 0.125 | 0.147 |
| Smad7 | 0.494* | 1.000** | 0.414 | 1 | -264 | -0.459 |
| YAP1 | 0.497* | -264 | 0.125 | -264 | 1 | 0.653** |
| MSTN | 0.096 | -459 | 0.147 | -459 | 0.653** | 1 |

In 6-month-old animals, the expression of *Smad2* was highly significantly and positively associated with *Smad3* expression in the gastrocnemius muscle (P < 0.01). The expression of *Smad3* was highly significantly (P < 0.01) and positively associated with *Smad4* expression in the gastrocnemius muscle (Table 4).

**Table 4.** Correlation between the expression of *Smads* and *YAP1* in the gastrocnemius dorsi muscle of 6-month-old animals.

| Groups | Smad2 | Smad3 | Smad4 | Smad7 | YAP1 | MSTN |
|---|---|---|---|---|---|---|
| Smad2 | 1 | 0.715** | 0.458 | 0.271 | -0.304 | -0.220 |
| Smad3 | 0.715** | 1 | 0.659** | 0.540* | -0.084 | 0.008 |
| Smad4 | 0.458 | 0.659** | 1 | 0.413 | 0.119 | -0.030 |
| Smad7 | 0.271 | 0.540* | 0.413 | 1 | 0.440 | 0.469* |
| YAP1 | -0.304 | -0.084 | 0.119 | 0.440 | 1 | 0.765** |
| MSTN | -0.220 | 0.008 | -0.030 | 0.469* | 0.765** | 1 |

The expression of *Smad2* was extremely significantly and positively associated with that of *Smad3*, *Smad4*, and *Smad7* (P < 0.01) in the gastrocnemius muscle at the three different growth stages. The expression of *Smad3* was highly significantly and positively associated with *Smad4* and *Smad7* (P < 0.01), and was highly significant and negative associated with *YAP1* (P < 0.01). The expression of *Smad4* was highly significant and positively associated with *Smad7* expression (P < 0.01). The expression of *Smad7* was not significant and was negatively (P > 0.05) associated with *YAP1* (Table 5).

**Table 5.** Correlation between the expression of *Smads* and *YAP1* in the gastrocnemius muscle.

| Groups | Smad2 | Smad3 | Smad4 | Smad7 | YAP1 | MSTN |
|---|---|---|---|---|---|---|
| Smad2 | 1 | 0.672** | 0.585** | 0.610** | -0.193 | -0.240 |
| Smad3 | 0.672** | 1 | 0.746** | 0.774** | -0.377** | -0.408** |
| Smad4 | 0.585** | 0.746** | 1 | 0.621** | -0.147 | -0.459** |
| Smad7 | 0.610** | 0.774** | 0.621** | 1 | -0.025 | -0.129 |
| YAP1 | -0.193 | -0.377** | -0.147 | -0.025 | 1 | 0.652** |
| MSTN | -0.240 | -0.408** | -0.459** | -0.129 | 0.652** | 1 |

At the 2-day-old stage, the expression of *Smad2* was highly significantly and positively associated with that of *Smad3*, *Smad4*, and *Smad7* in the extensor digitorum longus (P < 0.01). The expression of *Smad3* was highly significantly and positively associated with that of *Smad4*, *Smad7*, and *YAP1* in the extensor digitorum longus (P < 0.01). The expression of *Smad4* was highly significantly and positively (P < 0.01) associated with that of *Smad7* and *YAP1* in the extensor digitorum longus (Table 6).

**Table 6.** Correlation between the expression of *Smads* and *YAP1* in the extensor digitorum longus of 2-day-old animals.

| Groups | Smad2 | Smad3 | Smad4 | Smad7 | YAP1 | MSTN |
|---|---|---|---|---|---|---|
| Smad2 | 1 | 0.906** | 0.904** | 0.720** | 0.574* | 0.606* |
| Smad3 | 0.906** | 1 | 0.905** | 0.748** | 0.744** | 0.694** |
| Smad4 | 0.904** | 0.905** | 1 | 0.774** | 0.633* | 0.665** |
| Smad7 | 0.720** | 0.748** | 0.774** | 1 | 0.563* | 0.709** |
| YAP1 | 0.574* | 0.744** | 0.633* | 0.563* | 1 | 0.481* |
| MSTN | 0.606* | 0.694** | 0.665** | 0.709** | 0.481* | 1 |

In 2-month-old animals, the expression of *Smad2* was highly significantly and positively associated with that of *Smad3*, *Smad4*, and *Smad7* in the extensor digitorum longus (P < 0.01). The expression of *Smad3* was highly significantly and positively (P < 0.01) associated with that of *Smad7* in the extensor digitorum longus (Table 7).

**Table 7.** Correlation between the expression of *Smads* and *YAP1* in the extensor digitorum longus of 2-month-old animals.

| Groups | Smad2 | Smad3 | Smad4 | Smad7 | YAP1 | MSTN |
|---|---|---|---|---|---|---|
| Smad2 | 1 | 0.818** | 0.639** | 0.818** | 0.390 | 0.000 |
| Smad3 | 0.818** | 1 | 0.283 | 1.000** | 0.290 | -0.043 |
| Smad4 | 0.639** | 0.283 | 1 | 0.283 | 0.387 | 0.219 |
| Smad7 | 0.818** | 1.000** | 0.283 | 1 | 0.290 | -0.043 |
| YAP1 | 0.390 | 0.290 | 0.387 | 0.290 | 1 | 0.669** |
| MSTN | 0.000 | -0.043 | 0.219 | -0.043 | 0.669** | 1 |

In 6-month-old animals, the expression of *Smad2* was highly significantly and positively associated with that of *Smad3* and *Smad4* in the extensor digitorum longus (P < 0.01). Expression of *Smad3* was highly significantly and positively associated with that of *Smad4* in the extensor digitorum longus (P < 0.01). The expression of *Smad7* was not significant and was negatively (P > 0.05) associated with that of *YAP1* in the extensor digitorum longus (Table 8).

**Table 8.** Correlation between the expression of *Smads* and *YAP1* in the extensor digitorum longus of 6-month-old animals.

| Groups | Smad2 | Smad3 | Smad4 | Smad7 | YAP1 | MSTN |
|---|---|---|---|---|---|---|
| Smad2 | 1 | 0.740** | 0.876** | 0.575* | 0.303 | 0.429 |
| Smad3 | 0.740** | 1 | 0.777** | 0.390 | 0.203 | 0.218 |
| Smad4 | 0.876** | 0.777** | 1 | 0.575* | 0.424 | 0.320 |
| Smad7 | 0.575* | 0.390 | 0.575* | 1 | 0.701** | 0.760** |
| YAP1 | 0.303 | 0.203 | 0.424 | 0.701** | 1 | 0.585* |
| MSTN | 0.429 | 0.218 | 0.320 | 0.760** | 0.585* | 1 |

The expression of *Smad2* was highly significantly and positively associated with that of *Smad3*, *Smad4*, *Smad7*, and *YAP1* (P < 0.01) in the extensor digitorum longus at the three different growth stages. The expression of *Smad3* was highly significantly positively associated with that of *Smad4*, *Smad7*, and *YAP1* (P < 0.01). The expression of *Smad4* was highly significantly positively associated with that of *Smad7* and *YAP1* (P < 0.01). The expression of *Smad7* was highly significant and positively (P < 0.01) associated with that of *YAP1* (Table 9).

**Table 9.** Correlation between the expression of *Smads* and *YAP1* in the extensor digitorum longus.

| Groups | Smad2 | Smad3 | Smad4 | Smad7 | YAP1 | MSTN |
|---|---|---|---|---|---|---|
| Smad2 | 1 | 0.806** | 0.745** | 0.686** | 0.416** | 0.069 |
| Smad3 | 0.806** | 1 | 0.544** | 0.623** | 0.421** | 0.161 |
| Smad4 | 0.745** | 0.544** | 1 | 0.410** | 0.390** | 0.176 |
| Smad7 | 0.686** | 0.623** | 0.410** | 1 | 0.522** | 0.218 |
| YAP1 | 0.416** | 0.421** | 0.390** | 0.522** | 1 | 0.535** |
| MSTN | 0.069 | 0.161 | 0.176 | 0.218 | 0.535** | 1 |

## DISCUSSION

TGF-β regulates the growth and development of skeletal muscle through the recruitment of Smad proteins, which mediate the intracellular signaling of the TGF-β superfamily. As a transcriptional co-activator in the Hippo-YAP1 signaling pathway, YAP1 interacts with components of the TGF-β signaling pathway to co-regulate cellular behavior of the organism involving the transcription of Smad proteins. In our study, we investigated the expression of genes in the Smad family in the skeletal muscle of Hu sheep, and performed a correlation analysis with the muscle growth candidate gene YAP1. In this way, we explored the role of YAP1 in the regulation of muscle development in Hu sheep.

### Spatial and temporal expression of *Smads and YAP1*

Smad proteins are directly involved in TGF-β signaling transduction (Sekelsky et al., 1995; Savage et al., 1996; Zhang et al., 1996; Arai et al., 1998). In this study, we found that the expression of Smads in the extensor digitorum longus was lower than that in the gastrocnemius muscle, which may result from the different muscle fiber types. In different growth stages, the expression of *Smad2*, *Smad3*, and *Smad4* was higher at the 2-day-old stage than at any other stage (P < 0.05). Smad7 expression was lower in the 2-day-old animals than in the 6-month-old animals, and was the lowest in 2-month-old animals. The expression of *Smad2*, *Smad4*, and *Smad7* was higher in the 2-day-old males than in females, and was lower in the 2-month-old and 6-month-old males than in the 2-day-old females. *Smad3* gene expression was higher in 2-day-old and 2-month-old males than in females, but was lower in females at the 6-month-old stage. Xu (2010) showed that that the *Smad1* and *Smad4* genes were expressed in the hypothalamus, pituitary, ovary, heart, muscles, and other organs of Hu sheep. Hu et al. (2001) studied *Smad2* and *Smad4* expression in rat testes and found that *Smad2* was expressed in sperm cells and that *Smad4* was expressed in stromal cells. Cohen et al. (2015) found that increased Smad2/3 signaling in the absence of Smad7 inhibition impedes muscle growth and regeneration. Jueken (2014) found that the level of *Smad3* mRNA expression in the biceps femoris, semitendinosus, longissimus dorsi, and spleen of newborn lambs was significantly higher than that in the lung ($P < 0.05$). The levels of *Smad2* mRNA in the longissimus dorsi of 6-month-old lambs was significantly higher than in other tissues ($P < 0.05$), including fat, heart, lung, liver, spleen, and intestine ($P < 0.05$). These findings show that Smad2, Smad3, and Smad4 are expression in the muscle tissue, as well as in the visceral.

## Correlation analysis between *Smads* and *YAP1*

Correlation analysis of the expression of *Smads* and *YAP1* of sheep muscle revealed that the expressions of *YAP1* and *MSTN* were positively correlated ($P < 0.01$) in the gastrocnemius muscle of 2-day-old lambs. *YAP1* and *Smad2* was positively correlated ($P < 0.01$) in the gastrocnemius muscle of 2-month-old lambs. The expression of *YAP1* and *MSTN*, was extremely significantly positively correlated ($P < 0.01$) in the gastrocnemius muscle of 6-month-old lambs. The expression of *YAP1* was significantly positively correlated ($P < 0.01$) with that of *Smad4* and *Smad7* in the extensor digitorum longus of 2-day-old sheep. The expression of *YAP1* was negatively correlated with that of *Smad7*, although this was not significant. Highly significant differences between the expression of *Smad2* and *Smad3* ($P < 0.01$) in the two muscle tissues were found, which was also observed between *Smad3* and *Smad4*. This indicates that each of these genes has the same inhibitory effect on TGF-β/Smad signaling. In addition, there was a highly significant positive correlation ($P < 0.01$) between *YAP1* and *MSTN*, which suggested that *YAP1* may participate in regulating the TGF-β/Smad pathway and the process of muscle proliferation and differentiation.

## Conflicts of interest

The authors declare no conflict of interest.

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