Functional Analysis of Sheep POU2F3 Isoforms

Guang-Wei Ma¹² · Yan-Kai Chu¹² · Hua Yang³ · Xiao-Hong Yan¹² · En-Guang Rong⁴ · Hui Li¹² · Ning Wang¹²

Received: 25 May 2019 / Accepted: 17 December 2019 / Published online: 31 December 2019
© The Author(s) 2019

Abstract
POU domain class 2 transcription factor 3 (POU2F3) plays an important role in keratinocyte proliferation and differentiation. Our previous study identified four sheep POU2F3 transcript variants (POU2F3-1, POU2F3-2, POU2F3-3, and POU2F3-4), encoding three POU2F3 protein isoforms (POU2F3-1, POU2F3-2, and POU2F3-3). However, the functional differences among the three POU2F3 isoforms remain unknown. The objective of this study was to determine the tissue expression pattern of the four POU2F3 transcript variants in sheep and to investigate the functional differences in cell proliferation among the three POU2F3 isoforms. Quantitative RT-PCR analysis showed that the four POU2F3 transcripts were ubiquitously expressed in all tested adult sheep tissues, and POU2F3-1 exhibited higher expression level than the other three POU2F3 transcript variants in skin (P < 0.05). Cell proliferation assay showed that overexpression of any one of the three POU2F3 isoforms significantly inhibited the proliferation of sheep fetal fibroblasts and HaCaT cells at 48 and 72 h after transfection (P < 0.05). POU2F3-3 had less inhibitory effect on cell proliferation than POU2F3-1 and POU2F3-2 (P < 0.05), and POU2F3-1 and POU2F3-2 had similar inhibitory effects (P > 0.05). Dual luciferase reporter assays demonstrated that overexpression of any one of the three POU2F3 isoforms significantly inhibited the promoter activities of keratin 14 (KRT14) and matrix metalloproteinase 19 (MMP19) genes (P < 0.05). POU2F3-3 had less inhibitory effect on the promoter activities of KRT14 and MMP19 genes than POU2F3-1 and POU2F3-2 (P < 0.05), and POU2F3-1 and POU2F3-2 had similar inhibitory effects (P > 0.05). These results suggest three sheep POU2F3 isoforms have similar functional effects, but to a different extent.

Keywords POU2F3 · Isoforms · Cell proliferation · Promoter activity · Sheep
Introduction

POU domain class 2 transcription factor 3 (POU2F3), a POU domain transcription factor also known as Skn-1a or Oct-11, is predominantly expressed in the suprabasal layers of the epidermis (Goldsborough et al. 1993; Hildesheim et al. 2001). POU2F3 trans-activates the expression of keratinocyte differentiation marker genes, such as keratin 10 gene (KRT10) (Byrne et al. 1994) and small proline-rich protein 2A gene (SPRR2A) (Steinert and Marekov 1995), indicating that POU2F3 promotes keratinocyte differentiation (Takemoto et al. 2010). However, a previous study demonstrated that human POU2F3 (hPOU2F3) mainly promoted keratinocyte proliferation and secondarily enhanced keratinocyte differentiation (Hildesheim et al. 2001). In addition, a knockout study showed that POU2F3-knockout mice had higher proliferation rate of epidermal keratinocytes adjacent to the wound edge compared with the littermate controls (Andersen et al. 1997). A colony inhibition assay showed that human POU2F3 overexpression inhibited the proliferation of cervical cancer cell lines of epithelial origin (Yutaka et al. 2004). Keratin 14 gene (KRT14) and matrix metalloproteinase 19 genes (MMP19) are highly expressed in mitotically active epithelial basal cells and promote cell proliferation (Alam et al. 2011; Beck et al. 2007). POU2F3 overexpression inhibited the expression and promoter activity of the KRT14 gene in normal human epidermal keratinocytes (Sugihara et al. 2001) and of MMP19 gene in HaCaT cells (Beck et al. 2007; Sadowski et al. 2003).

Similar to other POU genes such as Oct-1, Oct-2, Brn-3, and Pit-1 (Ryan and Rosenfeld 1997), the POU2F3 gene can generate various transcript variants, with different expression patterns and functions. In rat epidermis, the Skn-1 gene generates two transcript variants with different functions, mSkn-1a and mSkn-1i (Andersen and Rosenfeld 1993). In human keratinocytes, Skn-1 gene produces three different transcript variants (hSkn-1a, hSkn-1d1 and hSkn-1d2) due to the alternative promoters, resulting in three proteins with different N-termini (hSkn-1a, hSkn-1d1 and hSkn-1d2) (Cabral et al. 2003). In our previous study, we identified four POU2F3 transcript variants: POU2F3-1, POU2F3-2, POU2F3-3, and POU2F3-4 (GenBank accession nos. JX184905, JX184906, JX184907, and JX184908), due to alternative splicing in Chinese Merino sheep, producing three protein isoforms with different N-termini (POU2F3-1, POU2F3-2, and POU2F3-3) (Rong et al. 2013). POU2F3-1 is the full-length POU2F3 (aa 1-435), POU2F3-2 (aa 34-435) lacks partial N-terminal 33 amino acids, and POU2F3-3 (aa 209-435) lacks the complete N-terminal and partial POU-specific domain (Rong et al. 2013). To date, the functional differences among the three POU2F3 isoforms remain unknown. In this study, we detected the tissue expression patterns of the four POU2F3 transcript variants in sheep and compared the effects of the three POU2F3 isoforms on the proliferation of sheep fetal fibroblasts (SFFs) and HaCaT cells, and on the promoter activities of KRT14 and MMP19 genes.
**Materials and Methods**

**Ethics Statement**

All animal work was carried out according to the guidelines for the care and use of experimental animals established by the Ministry of Science and Technology of the People’s Republic of China (Approval number: 2006-398) and approved by the Laboratory Animal Management Committee of Northeast Agricultural University.

**Animals and Tissue Collection**

Three rams from the superfine wool strain of Chinese Merino sheep (Junken Type), bred by the Xinjiang Academy of Agricultural and Reclamation Science were used for \( POU2F3 \) gene expression analysis. The 240-day-old sheep were slaughtered, and heart, liver, spleen, kidney, rumen, small intestine, skeletal muscle, and body side skin samples were collected. All collected tissue samples were snap-frozen in liquid nitrogen and stored at \(-80^\circ\text{C}\) for further analysis. The ear notch samples were collected during shearing. All sheep were kept in the same environment with free access to feed and water.

**Cell Culture**

HEK293 and HaCaT cells were purchased from the China Center for Type Culture Collection, and cultured in DMEM (Gibco). Sheep fetal fibroblasts (SFFs) as a kind gift from Dr. Tie-Zhu An, Northeast Forestry University, Harbin, were grown in DMEM-F12 (Gibco). Both DMEM and DMEM-F12 were supplemented with 10% FBS (Gibco) and 1% streptomycin/penicillin (Gibco). All cells were cultured in a humid environment with 5% CO\(_2\) in the air at 37\(^\circ\text{C}\).

**RNA Extraction and Quantitative RT-PCR Assay**

Total RNA of the frozen tissues or HaCaT cells was isolated using Trizol reagent (Invitrogen) according to the manufacturer’s instructions, and RNA quality was assessed by denaturing formaldehyde agarose gel electrophoresis. Reverse transcription of total RNA was performed using the Promega Improm-II reverse transcription System (Promega) according to the manufacturer’s instructions. Quantitative RT-PCR was carried out using SYBR Green PCR reagents on the 7500 real-time PCR system (Applied Biosystems) according to the manufacturer’s instructions. Quantitative RT-PCR was performed in triplicate for each sample. Sheep \( GAPDH \) or human \( GAPDH \) was used as the internal reference for the normalization of gene expression, and the relative mRNA expression was analyzed using \( 2^{-\Delta\Delta C_t} \) (Livak and Schmittgen 2001). The primers used for quantitative RT-PCR are shown in Table 1.
Plasmid Construction

The full-length CDS of the three sheep POU2F3 isoforms were amplified from the recombinant plasmids (pEASY-T1-POU2F3-1, pEASY-T1-POU2F3-2, pEASY-T1-POU2F3-3), respectively, which were previously generated in our laboratory (Rong et al. 2013), and cloned into the \textit{EcoR}I–\textit{Xho}I sites of the pCMV-Myc vector (Clontech), named pCMV-Myc-POU2F3-1, pCMV-Myc-POU2F3-2, and pCMV-Myc-POU2F3-3, respectively. For the promoter reporter plasmid construction, the 662-bp promoter fragment (−699 to −38 relative to the start codon ATG of sheep \textit{KRT14} gene) (Sugihara et al. 2001) and the 519-bp promoter fragment (−542 to −24 relative to the start codon ATG of sheep \textit{MMP19} gene) (IM et al. 2007) were amplified from the sheep genomic DNA (50 ng/μL), and subsequently cloned into the \textit{KpnI}–\textit{HindIII} sites of the pGL3-basic vector (Promega), named pGL3-basic-pKRT14 (−699/−38) and pGL3-basic-pMMP19 (−542/−24), respectively. All the constructions were confirmed by sequencing. The primers used for plasmid construction are listed in Table 2.

Western Blot

The coding potential of the three POU2F3 isoform expression plasmids (pCMV-Myc-POU2F3-1, pCMV-Myc-POU2F3-2, and pCMV-Myc-POU2F3-3) was verified by western blot. Briefly, HEK293 cells were seeded on 6-well plates with 1.2 × 10^6 cells/well. After overnight culture, the cells were transfected with pCMV-Myc-POU2F3-1, pCMV-Myc-POU2F3-2, or pCMV-Myc-POU2F3-3 using Lipofectamine 2000 reagent (Invitrogen). At 72 h after transfection, cells were washed twice with ice-cold PBS and lysed in RIPA Lysis Buffer (Beyotime) containing 10 μg/mL PMSF (Beyotime) for 30 min on ice. Then, the lysates were collected.
and centrifuged in Eppendorf tubes at 4 °C for 15 min. The equal amounts of protein from the cell lysates were re-suspended in gel sample buffer, separated by 10% SDS–polyacrylamide gel electrophoresis, and transferred to nitrocellulose membranes. The blots were blocked in PBS containing 5% (w/v) dry milk and 0.1% Tween 20 for 2 h and then incubated with primary antibody dilution buffer (Beyotime) containing Myc-tag mouse monoclonal antibody (Abcam, 1:1000) at room temperature for 2 h. After washing with PBS three times, the blots were incubated with a secondary antibody dilution buffer containing horseradish peroxidase-conjugated secondary antibody (1:5000) for 1 h, followed by washing with PBS for three times. Protein bands were visualized by chemiluminescence using the ECL kit (Sambrook et al. 1982).

**Cell Proliferation Assay**

Cell Counting Kit-8 (CCK-8) was used to measure cell proliferation. Briefly, 5 × 10⁴ SFFs or HaCaT cells were seeded in 96-well plates and transfected with pCMV-Myc-POU2F3-1, pCMV-Myc-POU2F3-2, pCMV-Myc-POU2F3-3, or pCMV-Myc using Lipofectamine 2000 reagent (Invitrogen). At 24, 48, and 72 h after transfection, each well was added with 10 μL CCK-8 solution and incubated at 37 °C for 2 h, and then the absorbance was recorded at 450 nm.

**Dual Luciferase Reporter Assay**

Dual luciferase reporter assay was performed in HEK293 cells. Briefly, 2.5 × 10⁵ cells were plated in 24-well plates, and co-transfected with the constructed POU2F3 expression vectors (pCMV-Myc-POU2F3-1, pCMV-Myc-POU2F3-2,
pCMV-Myc, or pCMV-Myc) and the constructed promoter reporters (pGL3-basic-pKRT14 (−699/−38) or pGL3-basic-pMMP19 (−542/−24) together with pRL-TK (Promega) using Lipofectamine 2000 reagent (Invitrogen). At 48 h of transfection, the relative luciferase activity was determined using the Dual-Glo Luciferase Assay System (Promega). Relative luciferase activity was obtained by the normalization to Renilla luciferase activity, known as the ratio of Firefly to Renilla luciferase. All luciferase assays were performed in triplicate. The data represented at least three independent experiments.

Statistical Analysis

All data were analyzed by ANOVA using the SPSS 19.0 software. \( P < 0.05 \) and \( P < 0.01 \) were considered statistically significant.

Results

Tissue Expression of POU2F3 Transcript Variants in Sheep

Our previous study showed that sheep \( POU2F3 \) gene produces four transcript variants (POU2F3-1, POU2F3-2, POU2F3-3, and POU2F3-4), encoding three POU2F3 protein isoforms (POU2F3-1, POU2F3-2, and POU2F3-3) (Rong et al. 2013). Of these four sheep \( POU2F3 \) transcript variants, POU2F3-1 contained all exons, but POU2F3-2 lacked exon 3, and POU2F3-3 lacked exons 4 and 5, and POU2F3-4 lacked exon 6 (Fig. S1). Based on their sequence characteristics, the transcript variant-specific primers were designed to detect tissue expression patterns of the four \( POU2F3 \) transcript variants in sheep using quantitative RT-PCR. The results showed that the four \( POU2F3 \) transcript variants were ubiquitously and differentially expressed in all tested adult sheep tissues including heart, liver, spleen, kidney, rumen, small intestine, skeletal muscle, and skin (Fig. 1). In the majority of the tested tissues, POU2F3-1 and POU2F3-2 were highly expressed, POU2F3-3 was intermediately expressed, and POU2F3-4 was low expressed. These four \( POU2F3 \) transcript variants were differentially expressed in the skin, and the expression levels of POU2F3-1, POU2F3-2 and POU2F3-3 were 87.60-, 14.54-, and 8.65-fold higher, respectively, than that of POU2F3-4 (\( P < 0.05 \); Fig. 1h).

Effects of Overexpression of POU2F3 Protein Isoforms on Cell Proliferation

It had been reported that human POU2F3 could promote keratinocyte proliferation (Hildesheim et al. 2001), however, the knockout mice study (Andersen et al. 1997) and the colony inhibition assay in cervical cancer cells (Yutaka et al. 2004) suggested POU2F3 might inhibit keratinocyte proliferation. To investigate whether sheep POU2F3 regulates cell proliferation and whether these three sheep POU2F3 isoforms have different effects on cell proliferation, we constructed the three POU2F3 isoform expression vectors (pCMV-Myc-POU2F3-1,
pCMV-Myc-POU2F3-2, and pCMV-Myc-POU2F3-3) and confirmed their expressions by western blot (Fig. 2a). These POU2F3 isoform expression plasmids were transiently transfected into either SFFs or HaCaT cells, and cell proliferation was assayed using CCK-8 assay. The results showed that the absorbance of both the SFFs and HaCaT cells transfected with any one of these POU2F3 isoform expression plasmids was significantly lower than that of the cells transfected with the empty vector pCMV-Myc at 48 and 72 h of transfection (\(P < 0.05\), Fig. 2b, c), suggesting that overexpression of any one of the three POU2F3 isoforms inhibits the proliferation of SFFs and HaCaT cells. POU2F3-1 and POU2F3-2 overexpression had a similar inhibitory effect on the cell proliferation (\(P > 0.05\), Fig. 2b, c), and both had greater inhibitory effects on the cell proliferation than POU2F3-3 in both SFFs and HaCaT cells at 72 h of transfection (\(P < 0.05\), Fig. 2b, c).

In parallel, we detected the expression of proliferation marker genes (\(Ki67\) and \(PCNA\)) using quantitative RT-PCR. Consistent with CCK-8 results, overexpression of any one of POU2F3 isoforms in SFFs cells, significantly inhibited \(Ki67\) expression compared with the empty vector pCMV-Myc at 48 h of transfection (\(P < 0.05\), Fig. 2d). POU2F3-1 and POU2F3-2 overexpression had a similar inhibitory effect on the cell proliferation (\(P > 0.05\), Fig. 2b, c), and both had greater inhibitory effects on the cell proliferation than POU2F3-3 in both SFFs and HaCaT cells at 72 h of transfection (\(P < 0.05\), Fig. 2b, c).

Similarly, \(PCNA\) expression was decreased when cells were transfected with each one of
three POU2F3 isoform expression vectors compared with the pCMV-Myc control (Fig. 2e). Taken together, these data suggested that these sheep POU2F3 isoforms inhibit cell proliferation, but to a different extent.
Effects of Overexpression of POU2F3 Protein Isoforms on the Promoter Activity of KRT14 and MMP19 Genes

Previous studies have demonstrated that POU2F3 down-regulated the promoter activities of KRT14 and MMP19 genes in HaCaT and HEK293 cells (IM et al. 2007; Sugihara et al. 2001). To reveal the functional differences among the three POU2F3 isoforms, we also investigated their effects on the promoter activity of KRT14 and MMP19 genes. The reporter gene assays showed that the cloned KRT14 and MMP19 promoters (pGL3-basic-pKRT14 (−699/−38) and pGL3-basic-pMMP19 (−542/−24)) were active compared with pGL3-basic vector (P < 0.05, Fig. 3a), and transfection of any one of the three POU2F3 isoform expression vectors significantly inhibited the promoter activities of KRT14 and MMP19 genes compared with the empty vector pCMV-Myc (P < 0.05, Fig. 3b, c). For KRT14 promoter, POU2F3-1 and POU2F3-2 showed similar inhibitory effects (P > 0.05, Fig. 3b), and they had greater inhibitory effects on the KRT14 promoter activity than POU2F3-3 (P < 0.05, Fig. 3b). For MMP19 promoter, POU2F3-1 had a greater inhibitory effect than POU2F3-2 and POU2F3-3 (P < 0.05, Fig. 3c), and POU2F3-2 had a greater inhibitory effect than POU2F3-3 (P < 0.05, Fig. 3c). Taken together, these data suggested that three sheep POU2F3 isoforms inhibit the promoter activities of KRT14 and MMP19 genes, but to a different extent.

![Fig. 3](image-url) Effects of three sheep POU2F3 isoforms on the promoter activities of KRT14 and MMP19 genes. a Luciferase reporter assay of sheep KRT14 promoter (−699/−38) and MMP19 promoter (−542/−24) in HEK293 cells at 48 h of transfection. Fold change was relative to the pGL3-basic at 48 h of transfection. b, c Effects of the three sheep POU2F3 isoforms on the promoter activities of KRT14 and MMP19 genes. Either of pGL3-basic-pKRT14 (−699/−38) or pGL3-basic-pMMP19 (−542/−24) and the indicated isoform expression vectors (pCMV-Myc-POU2F3-1, pCMV-Myc-POU2F3-2, or pCMV-Myc-POU2F3-3) were co-transfected into HEK293 cells. Forty-eight hours after transfection, cells were lysed and luciferase activity was measured. Results were normalized with the Renilla luciferase activity, and expressed as the ratio of Firefly to Renilla luciferase. Fold change was relative to the empty vector pCMV-Myc at 48 h of transfection. All data are representative of three independent experiments and are shown as the mean ± SEM. For each figure panel, different letters above error bars indicate a statistically significant difference (P < 0.05)
Discussion

Alternative splicing is a crucial mechanism of regulation of gene expression and protein diversity (Barmak and Christopher 2001; Stamm et al. 2013). High-throughput sequencing has revealed that 92–94% of human genes undergo alternative splicing (Qun et al. 2008; Wang et al. 2008). Alternative splicing generates protein isoforms with different or even opposite functions (Stamm et al. 2013). Our previous finding showed that sheep POU2F3 produces four mRNA transcript variants due to alternative splicing, and encodes three protein isoforms (POU2F3-1, POU2F3-2, and POU2F3-3) with various N terminus (Rong et al. 2013). In the present study, our results showed that four sheep POU2F3 transcript variants were widely and differentially expressed in various tissues of the Chinese Merino sheep, especially the skin (Fig. 1), suggesting that POU2F3 alternative splicing is regulated in a tissue-specific manner and that these POU2F3 isoforms might exert different functions. Consistently, we found that these three sheep POU2F3 protein isoforms inhibited the proliferation of SFFs and HaCaT cells, but to a different extent (Fig. 2b, c).

In agreement with our results, several reports have indicated that POU2F3 inhibits cell proliferation (Andersen et al. 1997; Sadowski et al. 2003; Sugihara et al. 2001; Yutaka et al. 2004). However, human POU2F3 has been shown to promote keratinocyte proliferation (Hildesheim et al. 2001). This difference suggests that POU2F3 may play different roles in cell proliferation, depending on cell type, cellular context, and species.

KRT14 and MMP19 genes are two target genes of POU2F3, highly expressed in mitotically active epithelial basal cells, and their expressions are down-regulated during cell differentiation (Beck et al. 2007; Sugihara et al. 2001). In HaCaT cells, KRT14 knockdown inhibited cell proliferation (Alam et al. 2011), while MMP19 overexpression increased cell proliferation (IM et al. 2007). These data indicate that KRT14 and MMP19 are proliferation-promoting genes. Consistent with this notion, our results showed that the three sheep POU2F3 isoforms inhibited the promoter activities of KRT14 and MMP19 genes (Fig. 3b, c) and the proliferation of SFFs and HaCaT cells to a different extent (Fig. 2b, c). Combined with these data, it is reasonable to speculate that sheep POU2F3 might inhibit cell proliferation by down-regulating KRT14 and MMP19 genes.

The POU class of transcription factors is characterized by the POU-specific domain located in the upstream of a POU-homeodomain (Bürglin and Affolter 2016). POU-homeodomain is responsible for DNA-binding (Klemm et al. 1994) and protein–protein interaction (Cabral et al. 2003). The POU-specific domain is also involved in DNA-binding. This domain binds to DNA in two conformations (Reményi et al. 2001). In one conformation, POU-specific domain binds to a 15-bp DNA sequence called PORE, which is not palindromic. In another conformation, it binds to a 12-bp palindromic DNA sequence, named MORE (Bürglin and Affolter 2016). The previous study showed that hSkn-1a containing all coding exons with amino acids of 1–430 repressed the hKRT14 promoter activity by about threefold in normal human epidermal keratinocytes (P <0.05).
(Sugihara et al. 2001). Interestingly, ΔC-hSkn-1a lacking C-terminal repressed the hKRT14 promoter activity to the same extent as hSkn-1a (P > 0.05), however, ΔN-hSkn-1a removing N-terminal significantly decreased the inhibitory effect compared with hSkn-1a (P < 0.05) (Sugihara et al. 2001). These results suggest that the POU-specific domain and POU-homeodomain are required for the inhibitory effect of hSkn-1a and ΔC-hSkn-1a on the hKRT14 promoter activity, and the full repression requires the complete N-terminal. Consistent with this suggestion, our study showed that all these three sheep POU2F3 isoforms inhibited the promoter activities of KRT14 and MMP19 genes to a different extent. Their inhibitory effects might be explained by their shared POU-homeodomain (Rong et al. 2013), and the extent of different inhibition might be due to their differences in the N-terminal and POU-specific domain (Rong et al. 2013). It is worth investigating the molecular mechanisms underlying the functional differences among the three POU2F3 isoforms in the future.

Conclusion

This is the first report showing the functional differences among sheep POU2F3 isoforms. We demonstrated that the four sheep POU2F3 transcripts were ubiquitously and differentially expressed in adult Chinese Merino sheep tissues, and the three sheep POU2F3 protein isoforms inhibited the proliferation of SFFs and HaCaT cells and the promoter activities of KRT14 and MMP19 to a different extent.

Acknowledgements

This study was funded by Domain-Specific projects for transgenic biological breeding (Grant Nos. 2014ZX08009-002 and 2009ZX08009-160B).

Conflict of interest

The authors declare that they have no conflict of interest.

Open Access

This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit https://creativecommons.org/licenses/by/4.0/.

References

Alam H, Sehgal L, Kundu ST, Dalal SN, Vaidya MM (2011) Novel function of keratins 5 and 14 in proliferation and differentiation of stratified epithelial cells. Mol Biol Cell 22:4068
Andersen B, Rosenfeld MG (1993) Skn-1a and Skn-1i: two functionally distinct Oct-2-related factors expressed in epidermis. Science 260:78–82
Andersen B, Weinberg WC, Rennekampff O, Mcevilly RJ, Birmingham BJ Jr, Hooshmand F, Vasilyev V, Hansbrough JF, Pittelkow MR, Yuspa SH (1997) Functions of the POU domain genes Skn-1a/i and Tst-1/Oct-6/SCIP in epidermal differentiation. Genes Dev 11:1873–1884

Barmak M, Christopher L (2001) A genomic view of alternative splicing. Nat Genet 30:13–19

Beck IM, Müller M, Mentlein R, Sadowski T, Mueller MS, Paus R, Sedlacek R (2007) Matrix metalloproteinase-19 expression in keratinocytes is repressed by transcription factors Tst-1 and Skn-1a: implications for keratinocyte differentiation. J Investig Dermatol 127:1107

Bürglin TR, Affolter M (2016) Homeodomain proteins: an update. Chromosoma 125:497–521

Byrne C, Tainsky M, Fuchs E (1994) Programming gene expression in developing epidermis. Development 120:2369–2383

Cabral A, Fischer DF, Vermeij WP, Backendorf C (2003) Distinct functional interactions of human Skn-1 isoforms with Ese-1 during keratinocyte terminal differentiation. J Biol Chem 278:17792–17799

Goldsborough AS, Healy LE, Copeland NG, Gilbert DJ, Jenkins NA, Willison KR, Ashworth A (1993) Cloning, chromosomal localization and expression pattern of the POU domain gene Oct-11. Nucleic Acids Res 21:127–134

Hildesheim J, Kühn U, Yee CL, Foster RA, Yancey KB, Vogel JC (2001) The hSkn-1a POU transcription factor enhances epidermal stratification by promoting keratinocyte proliferation. J Cell Sci 114:1913–1923

Klemm JD, Rouil MA, Aurora R, Herr W, Pabo CO (1994) Crystal structure of the Oct-1 POU domain bound to an octamer site: DNA recognition with tethered DNA-binding modules. Cell 77:21–32

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(−Delta Delta C(T)) method. Methods 25:402–408

Qun P, Ofer S, Lee LJ, Frey BJ, Blencowe BJ (2008) Deep surveying of alternative splicing complexity in the human transcriptome by high-throughput sequencing. Nat Genet 40:1413–1415

Reményi A, Tomilin A, Pohl E, Lins K, Philippesen A, Reinbold R, Schöler HR, Wilmanns M (2001) Dif- ferential dimer activities of the transcription factor Oct-1 by DNA-induced interface swapping. Mol Cell 8:569–580

Rong EG, Qiao SP, Lei YU, Yan XH, Yang H, Hui LI, Wang N (2013) Cloning and expression characterization of sheep POU2F3 gene and its isoforms in chinese merino skin. Chin J Anim Vet Sci 44:1189–1197

Ryan AK, Rosenfeld MG (1997) POU domain family values: flexibility, partnerships, and developmental codes. Genes Dev 11:1207

Sadowski T, Dietrich S, Koschinsky F, Sedlacek R (2003) Matrix metalloproteinase 19 regulates insulin-like growth factor-mediated proliferation, migration, and adhesion in human keratinocytes through proteolysis of insulin-like growth factor binding protein-3. Mol Biol 14:4569–4580

Sambrook J, Fritsch EF, Maniatis T (1982) Molecular cloning: a laboratory manual. Cold Spring Harbor, Cold Spring Harbor Laboratory

Stamn S, Benari S, Rafalska I, Tang Y, Zhang Z, Toiber D, Thanaraj TA, Soreq H (2013) Function of alternative splicing. Gene 514:1–30

Steinert PM, Marekov LN (1995) The proteins elafin, filaggrin, keratin intermediate filaments, loricrin, and small proline-rich proteins 1 and 2 are isodipeptide cross-linked components of the human epidermal cornified cell envelope. J Biol Chem 270:17702

Sugihara TM, Kudryavtseva EI, Kumar V, Horridge JJ, Andersen B (2001) The POU domain factor Skin-1a represses the keratin 14 promoter independent of DNA binding: A possible role for interactions between Skn-1a and CREB-binding protein/p300. J Biol Chem 276:33036–33044

Takemoto H, Tamai K, Akasaka E, Rokunohe D, Takiyoshi N, Umegaki N, Nakajima K, Aizu T, Kaneko T, Nakano H (2010) Relation between the expression levels of the POU transcription factors Skn-1a and Skn-1n and keratinocyte differentiation. J Dermatol Sci 60:203–205

Wang ET, Rickard S, Shujun L, Irina K, Lu Z, Christine M, Kingsmore SF, Schroth GP, Burge CB (2008) Alternative isoform regulation in human tissue transcriptomes. Nature 456:470–476

Yutaka E, Kikuko E, Tadaichi K, Tadahito K (2004) Keratinocyte-specific POU transcription factor hSkn-1a represses the growth of cervical cancer cell lines. Oncogene 23:5014–5022

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.
Affiliations

Guang‑Wei Ma1,2 · Yan‑Kai Chu1,2 · Hua Yang3 · Xiao‑Hong Yan1,2 · En‑Guang Rong4 · Hui Li1,2 · Ning Wang1,2

Guang‑Wei Ma
maguangwei77@163.com

Yan‑Kai Chu
845039965@qq.com

Hua Yang
465911018@qq.com

Xiao‑Hong Yan
yanxiaohong@neau.edu.cn

En‑Guang Rong
453886782@qq.com

Hui Li
lihui@neau.edu.cn

1 College of Animal Science and Technology, Northeast Agricultural University, Harbin 150030, People’s Republic of China
2 Key Laboratory of Chicken Genetics and Breedings, Ministry of Agriculture and Rural Affairs, Harbin 150030, People’s Republic of China
3 Institute of Animal Husbandry and Veterinary Medicine, Xinjiang Academy of Agricultural and Reclamation Science, Shihezi 832000, People’s Republic of China
4 State Key Laboratory of Agrobiotechnology, China Agricultural University, Beijing 100193, People’s Republic of China