The Landscape of Chromatin Accessibility in Skeletal Muscle During Embryonic Development in Pigs

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Research

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Abstract

Background: The development of skeletal muscle during the embryonic stage in pigs is precisely regulated by transcriptional regulation, which depends on chromatin accessibility. However, how chromatin accessibility plays a regulatory role during embryonic skeletal muscle development in pigs has not been reported. To gain insight into the landscape of chromatin accessibility and the associated genome-wide transcriptome during embryonic muscle development, we performed ATAC-seq and RNA-seq on skeletal muscle of pig embryos at 45, 70 and 100 days post coitus (dpc).

Results: In total, 21638, 35447 and 60181 unique regions (or peaks) were found across 45 dpc (LW45), 70 dpc (LW70) and 100 dpc (LW100) embryos, respectively. More than 91% of peaks were annotated within -1 kb to 100 bp of transcription start sites (TSSs). First, widespread increases in specific accessible chromatin regions (ACRs) from 45 to 100 dpc embryos suggested that the regulatory mechanisms became increasingly complicated during embryonic development. Second, the findings of integrated ATAC-seq and RNA-seq analyses showed that not only the numbers but also the peak intensities of ACRs could control the expression of associated genes. Finally, motif screening of stage-specific ACRs revealed some transcription factors that regulated muscle development-related genes, such as MyoD, Mef2c, Mef2d and Pax7. Several potential transcriptional repressors, including E2F6, GRHL2, OTX2 and CTCF, were identified among those genes that exhibited different change trends between the ATAC-seq and RNA-seq data.

Conclusions: This work indicates that chromatin accessibility plays an important regulatory role in the embryonic muscle development of pigs and regulates the temporal and spatial expression patterns of key genes in muscle development by influencing the binding of transcription factors. Our results contribute to a better understanding of the regulatory dynamics of genes involved in pig embryonic skeletal muscle development.

Full Text

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Figures

Figure 1

Overview of the ATAC-seq libraries. (Schematic of the experimental design. B) Distribution s of ACR numbers across different chromosomes . C) Percentage s of ACRs in different genomic regions. (D F) Distributions of ACR lengths in different genomic regions. Different letters above violin s indicate significant differences between the groups based on Tukey's honestly significant difference test $P < 0.05$).

Figure 2

Expression levels of genes associated with ACRs. (A) Statistics for ACR numbers in the proximal promoter regions of genes. (B) Heatmap of the percentages of ACR-associated gene expression levels in different groups. associated gene expression levels in different groups. ""One ACR One ACR"" and "">one >one ACRACR"" correspond correspond to the groups to the groups in which in which genes contained one ACR and multiple genes contained one ACR and multiple ACRs in their proximal promoter

region ACRs in their proximal promoter regions s, respectively. , respectively. T The expression level he expression levels s of genes of genes were were divided into six groups divided into six groups based based on on the the FPKM FPKM values: 0values: 0--1, 11, 1--2, 22, 2--5, 55, 5--10, 1010, 10--30, 30, 734 and >30and >30. (. (CC-E)E) P Percentage percentages s of ACR of ACR-associated gene expression levels in different associated gene expression levels in different 735 groups. The groups. The genes were grouped according to whether they contained a single ACR genes were grouped according to whether they contained a single ACR 736 ((upper upper panel) panel) or or multiple ACRs (multiple ACRs (middle middle and and lower lower panel panel)) in their proximal promoters in their proximal promoters 737 according to the peak length according to the peak length ((middle middle panel panel: base: based d on longest peak length; on longest peak length; lower lower panel panel: : 738 base based d on total length)on total length) and and were were then divided into 3 equal then divided into 3 equal groups (the top, middle and groups (the top, middle and 739 bottom groups bottom groups)).).

Figure 3

ACR changes during muscle development. (A) Visualization of ACRs in the LW45, LW70 and LW100 groups within a region on Chr1. (B) Statistics for common and specific ACRs in the LW45, LW70 and LW100 groups. (C) Visualization of specific ACRs at different stages. (D) Visualization of common ACRs in MYOD1 at different stages. (E) Heatmap of the expression of XRCCI, ENPP2, BCL6 and MYOD1 at the LW 45, LW 70 and LW 100 stages.

Figure 4

Functional analysis of genes associated with stage specific ACR s. (A) 749 Number s of genes related to stage specific ACR s. B) BPs of the top 15 genes in the 750 LW45, LW70 and LW100 groups. The dot color represents the statistical significance 751 level of enrichment (p adjust); the dot size indicate s the proportion of genes enriched for the corresponding the corresponding term terms. (s. (C) C) KEGKEGG pathways of G pathways of the the top 15 top 15 genes genes in in the the LW45, LW45, 753 LW70 and LW100 groups. LW70 and LW100 groups. The dot color represents the statistical significance The dot color represents the statistical significance level level of of 754 enrichment (enrichment (p. adjust p. adjust); the dot size); the dot size indicate indicates the proportion of genes s the proportion of genes enriched enriched for for the the 755 corresponding corresponding term terms. (s. (D)D) Number Numbers

s of shared and stage of shared and stage-specific genes associated with specific genes associated with 756 specific ACRs in specific ACRs in the the LW45, LW70 and LW100LW45, LW70 and LW100 groups groups. (. (E)E) GO analysis of shared GO analysis of shared 757 genes in genes in the the LW45, LW70 and LW100LW45, LW70 and LW100 groups groups.. 758 759

Figure 5

Functional analysis of genes associated with stage specific ACR s. (A) Numbers of genes with DPs in ACRs shared among stages . B) The BPs of the top 15 genes in the LW100 vs LW70 and LW100 vs LW45 comparisons . The dot color represents the statistical significance level of enrichment (p. adjust); the dot size indicate s the proportion of genes enriched for the corresponding term s. (C) KEGG pathways of the top 15 genes in the LW70 vs LW45, LW100 vs LW70 and LW100 vs LW45 comparisons . The dot color represents the statistical significance level of enrichment (p. adjust); the dot size indicate s the proportion of genes enriched for the corresponding term s. (D) Number s of shared and stage specific genes associated with common ACRs in the LW70 vs LW45, LW100 vs LW70 and LW100 vs LW45 groups.

Figure 6

Identification of regulatory DNA elements at different stages . (Heatmap of the expression levels of DEGs at different developmental stages . B) Expression profile s of all DEGs in six clusters. (C) Statistics for the overlap between genes with DPIs and DEGs. D) TF motifs identified in the different groups.

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