Transcriptomic profiling comparison of YAP over-expression and conditional knockout mouse tooth germs

Ming Liu, Xiu-Ping Wang *

Department of Developmental Biology, Harvard School of Dental Medicine, Boston, MA 02115, USA

Abstract

To identify the downstream target genes of YAP, we used RNA-Seq technology to compare the transcriptomic profiling of Yap conditional knockout (Yap CKO) and YAP over-expression mouse tooth germs. Our results showed that some Hox, Wnt and Laminin family genes had concurrent changes with YAP transcripts, indicating that the expression of these genes may be regulated by YAP. Here, we provide the detailed experimental procedure for the transcriptomic profiling results (NCBI GEO accession number GSE65524). The associated study on the regulation of Hoxa1 and Hoxc13 genes by YAP was published in Molecular Cellular Biology in 2015 [Liu et al., 2015].

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1. Direct link to deposited data

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE65524.

2. Experimental design, materials and methods

2.1. Animal usage

The human keratin 14 promoter (K14) was used to either conditionally knockout Yap or drive YAP transgenic over-expression in mouse embryonic ectoderm-derived epithelial tissues. Yap conditional knockout (CKO) embryos were generated through crossing Yap<sup>fl/fl</sup> mice with K14-Cre mice [1,2] in C57BL/6 background. YAP over-expression (OE) embryos were generated through breeding Col-TetO-YAPS127A mice with K14-rtTA mice [1–5] in FVB/NJ background, in which the constitutively active form of human YAP1 protein with the Ser127Ala mutation was over-expressed upon Doxycycline (Dox) administration. 2 mg/ml of Dox in drinking water was given starting from embryonic days 9.5 (E9.5) to E14.5. All mouse studies were performed in compliance with the protocols approved by the Harvard University Institutional Animal Care and Use Committee.

2.2. E14.5 embryonic tooth germ dissection, collection and RNA preparation

Three Yap CKO embryos at E14.5, along with the three corresponding litter controls, were used for tooth germ collection of the Yap CKO group. Similarly, three YAP over-expression (OE) embryos were generated through breeding Col-TetO-YAPS127A mice with K14-rtTA mice [1–5] in FVB/NJ background, in which the constitutively active form of human YAP1 protein with the Ser127Ala mutation was over-expressed upon Doxycycline (Dox) administration. 2 mg/ml of Dox in drinking water was given starting from embryonic days 9.5 (E9.5) to E14.5. All mouse studies were performed in compliance with the protocols approved by the Harvard University Institutional Animal Care and Use Committee.

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2.3. RNA-sequencing

RNA-sequencing was performed on an illumina HiSeq 2000 by the Biopolymers Facility at Harvard Medical School. Paired end 50-nt sequencing strategy was used for all the RNA-Seq samples to minimize sequencing reading errors.

2.4. Data analysis

Mus musculus genome (mm9) and DNAnexus software were used to extract sequencing reads of genes from the RNA-Seq raw data. RNA-Seq raw sequencing reads and aligned reads are available through the Gene Expression Omnibus at the accession number of GSE65524. After RNA-Seq reads were extracted and aligned with the mm9 genome, the relative read ratio of each gene was calculated and further analysis was conducted. We compared the transcriptomic profilings between the Yap CKO and wild type tooth germs, as well as those between the YAP OE and wild type mouse tooth germs. In comparison with the corresponding controls at the 1.5-fold change cutoff, there were 968 down-regulated genes and 979 up-regulated genes in Yap CKO tooth germs, while there were down-regulated 1289 genes and up-regulated 774 genes in YAP over-expression tooth germs (Fig. 2). Further analysis revealed many differentially expressed genes between Yap CKO and YAP OE tooth germs. Interestingly, we found that some genes in the Hox, Wnt and Laminin families were differentially expressed in the two kinds of the tooth germs. The transcription levels of several Hox gene family members were decreased in Yap CKO tooth germs while their expression levels were increased in YAP OE tooth germs (Fig. 3), indicating that these genes could be potential downstream targets of YAP in vivo. The regulation of Hoxa1 and Hoxc13 genes by YAP was further validated and functionally analyzed in different epithelial cells of mouse tooth germs, skin samples and human keratinocytes [1].

The transcriptomic profiling comparison revealed that Hoxa1, Hoxa2, Hoxa3, Hoxa5, Hoxb9, Hoxc13, Hoxc4, Hoxc8 and Hoxd1 have differential expression in Yap CKO and YAP OE tooth germs. However, our qPCR results showed that only Hoxa1 and Hoxc13 had significant concurrent changes in their transcripts. This discrepancy could be due to low abundance of the Hox genes in mouse tooth tissues, which may cause high relative ratios.

Previous studies demonstrated that Wnt/β-catenin signaling regulates YAP expression in vitro [6,7]. However, how YAP affects the Wnt family members remains unknown. There are also some other gene families showing concurrent transcript changes with YAP transcripts, such as the Laminin, Rho GTPases activating protein and Ras-related

3. Discussion

In this study, we used RNA-Seq analysis to compare the transcriptomic profilings of Yap CKO and YAP over-expression mouse tooth germs. We found that some genes in the Hox, Wnt and Laminin families exhibiting concurrent changes with YAP transcripts and may be potentially targets of YAP. The regulation of Hoxa1 and Hoxc13 genes by YAP was further validated and functionally analyzed in different epithelial cells of mouse tooth germs, skin samples and human keratinocytes [1].

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![Fig. 1. Quality control of the total RNA samples of Yap CKO and YAP OE tooth germs. (A) Electrophoresis of the total RNA samples. (B) RNA integrity numbers of the total RNA samples. The Yap CKO samples: 1, 2 and 3; the Yap CKO control samples: 6, 7 and 8; the YAP OE samples: 7, 11 and 12; the YAP OE control samples: 8, 9 and 10.](image1)

![Fig. 2. The down-regulated and up-regulated gene numbers in the Yap CKO and YAP OE tooth germs at the 1.5-fold cutoff in the RNA-Seq data.](image2)
protein gene family members. Further investigation is required to understand the regulation of their expression by YAP and the relationships among these gene families.

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