MCB
Journals.ASM.org

YAP Regulates the Expression of *Hoxa1* and *Hoxc13* in Mouse and Human Oral and Skin Epithelial Tissues

Ming Liu,[a] Shuangyun Zhao,[a,b] Qingjie Lin,[a,c] Xiu-Ping Wang[a]

Department of Developmental Biology, Harvard School of Dental Medicine, Boston, Massachusetts, USA[a]; Department of Pediatric Dentistry, School of Stomatology, Peking University, Beijing, China[b]; Department of Implantation, School of Stomatology, Shandong University, Jinan, China[c]

Yes-associated protein (YAP) is a Hippo signaling transcriptional coactivator that plays pivotal roles in stem cell proliferation, organ size control, and tumor development. The downstream targets of YAP have been shown to be highly context dependent. In this study, we used the embryonic mouse tooth germ as a tool to search for the downstream targets of YAP in ectoderm-derived tissues. *Yap* deficiency in the dental epithelium resulted in a small tooth germ with reduced epithelial cell proliferation. We compared the gene expression profiles of embryonic day 14.5 (E14.5) *Yap* conditional knockout and *YAP* transgenic mouse tooth germs using transcriptome sequencing (RNA-Seq) and further confirmed the differentially expressed genes using real-time PCR and *in situ* hybridization. We found that YAP regulates the expression of *Hoxa1* and *Hoxc13* in oral and dental epithelial tissues as well as in the epidermis of skin during embryonic and adult stages. Sphere formation assay suggested that *Hoxa1* and *Hoxc13* are functionally involved in YAP-regulated epithelial progenitor cell proliferation, and chromatin immunoprecipitation (ChIP) assay implies that YAP may regulate *Hoxa1* and *Hoxc13* expression through TEAD transcription factors. These results provide mechanistic insights into abnormal YAP activities in mice and humans.

Yes-associated protein (YAP) is a key transcriptional coactivator of the Hippo signaling pathway that plays pivotal roles in stem/progenitor cell proliferation and organ size control (1–11). YAP has also been shown to be a candidate oncogene in the development and progression of multiple human cancers (12–14). The activity of YAP is negatively regulated by its upstream kinase cascade (MstI/2, Sav1, Lats1/2, and Mob1), which leads to the phosphorylation and subsequent degradation of YAP and its paralog TAZ. Inhibition of Hippo signaling relieves YAP and TAZ, which can then translocate into the nucleus. In the nucleus, YAP or TAZ associates with TEAD or other transcription factors to activate the transcription of its target genes (15–18). Conventional knockout of *Yap* in mice causes early embryonic lethality due to defects in yolk sac vasculogenesis (19). Overexpression of YAP results in enlarged organ size in *Drosophila* and in mice with profound cell proliferation and inhibition of apoptosis (1, 2, 7, 11, 20). In addition, YAP also plays a critical role in maintaining mouse embryonic stem cell pluripotency and regulating tissue-specific progenitor cells (21).

Although the core components of the Hippo pathway are highly conserved between *Drosophila* and mammalian systems, the transcriptional outputs differ greatly depending on when and where the pathway is deployed. For example, overexpression of YAP in the mouse small intestine leads to Notch-dependent hyperplasia and loss of terminally differentiated cell types but does not appreciably increase the overall size of the organ (1). In *Drosophila*, the YAP ortholog Yki induces the expression of *cycE*, *diap1*, and *bantam* microRNA (11, 22). In mammalian cells, YAP induces *Birc2* and *Birc5*, two *diap1* homologs, and *connective tissue growth factor* (*CTGF*), *Amphiregulin* (*AREG*), and *Cyr61* (2, 15, 20, 23, 24). Most YAP targets appear to be tissue and cell type specific, suggesting that the function of YAP in mammals is modulated by various biological inputs and associated proteins in a context-dependent manner.

Teeth are typical ectodermal appendages that develop through sequential and reciprocal interactions between oral epithelium and the underlying neural-crest-derived mesenchyme. The early stage of tooth development morphologically and molecularly resembles that of other ectodermal organs, such as hair, feathers, and salivary glands (25). We previously reported that *Yap* is expressed in both dental epithelial and mesenchymal tissues, with slightly elevated expression in the inner and outer dental epithelia. Overexpression of YAP in the dental epithelium affects tooth morphogenesis and the patterning of enamel knots, while the signaling center remains induced at the tip of the tooth germ by epithelium-mesenchyme interactions (26). In this study, we compared the gene expression profiles of embryonic day 14.5 (E14.5) *Yap* conditional knockout (CKO) and *YAP* transgenic (Tg) mouse tooth germs using transcriptome sequencing (RNA-Seq) analysis and further confirmed the differentially expressed genes using real-time PCR, *in situ* hybridization, sphere formation assay, and chromatin immunoprecipitation (ChIP) assay. We found that YAP regulates the expression of *Hoxa1* and *Hoxc13* in oral and dental epithelial tissues as well as in the epidermis of skin during the embryonic and adult stages. The regulation of *Hoxa1* and *Hoxc13* by YAP is highly conserved between mice and humans and may be mediated through the TEAD transcription factors. Since misregulation of YAP, HOXA1, and HOXC13 can cause various developmental disorders and human cancers, these results provide insight into the molecular mechanisms underlying abnormal

Received 4 June 2014 **Returned for modification** 10 July 2014
Accepted 5 February 2015
Accepted manuscript posted online 17 February 2015
Citation Liu M, Zhao S, Lin Q, Wang X-P. 2015. YAP regulates the expression of *Hoxa1* and *Hoxc13* in mouse and human oral and skin epithelial tissues. Mol Cell Biol 35:1449–1461. doi:10.1128/MCB.00765-14.
Address correspondence to Xiu-Ping Wang, xwang2527@gmail.com.

doi:10.1128/MCB.00765-14

YAP activities in mice and humans and may also provide clues for potential treatment targets.

MATERIALS AND METHODS

Animals. All mouse use was in compliance with the protocols approved by Harvard University Institutional Animal Care and Use Committee. *Yap* conditional knockout genes and *YAP* transgenes were under the control of the human keratin 14 (K14) promoter, which drives gene expression in ectoderm-derived epithelial tissues. *Yap* CKO mice were generated through breeding $Yap^{fl/fl}$ and *K14-Cre* mice (27). *YAP* Tg mice were generated through breeding *Col-TetO-*YAP^{S127A} mice with *K14-rtTA* mice (1, 27, 28) in which a human YAP1 protein with a mutation in residue 127 (Ser → Ala) was constitutively activated upon doxycycline (Dox) administration (2 mg/ml in drinking water).

Histology, apoptosis assay, and cell proliferation analysis. Embryonic mouse heads and adult skin samples were fixed in 4% paraformaldehyde (PFA)–phosphate-buffered saline (PBS), dehydrated using an ethanol series, embedded in paraffin, sectioned at intervals of 7 to 10 μm, and stained with hematoxylin and eosin (HE) for morphological examination. Fluorescence images were acquired using conventional microscopes (Zeiss Axio) or confocal fluorescence microscopes (Zeiss LSM510). Apoptosis was assessed by terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling (TUNEL) staining (Roche), and cell proliferation was examined using an EdU (5-ethynyl-2′-deoxyuridine) incorporation staining kit (Life Technologies) in serial sections of E14.5 mouse tooth germs according to the manufacturer's protocol.

Cell culture, YAP knockdown, and overexpression in human keratinocytes. HaCaT cells (an immortalized human keratinocyte line) were purchased from Cell Line Service (CLS, Germany). HaCaT cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and penicillin-streptomycin, transfected with scrambled control small interfering RNA (siRNA) or YAP siRNA using Lipofectamine RNAiMAX (Life Technologies), and collected at 24-h, 48-h, and 72-h intervals for total RNA extraction. YAP^{S127A}-inducible HaCaT cells (HaCaT-iYAP^{S127A}) were cultured in CNT-07 defined epidermal keratinocyte medium (CELLnTEC, Switzerland), treated with Dox (100 ng/ml), and collected at 6-h, 24-h, and 48-h intervals for total RNA extraction (27).

Sphere formation assay. Growth factor reduced Matrigel (BD Biosciences) was used to coat eight-well chamber slides (BD Falcon CultureSlide). HaCaT-iYAP^{S127A} cells (5,000 per well) were seeded in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 2% Matrigel, and penicillin-streptomycin overnight and were then treated with scrambled control siRNA, *YAP* siRNA, *HOXA1* siRNA, or *HOXC13* siRNA overnight. After that, Dox (100 ng/ml) was added to CNT-BM media (CELLnTEC) containing 2% Matrigel to induce *YAP* expression. The medium was changed every 3 days, and the number of spheres in each well was counted after 10 days of culture.

RNA-Seq analysis and qPCR. Total RNA was extracted and purified using TRIzol (Life Technologies) and an RNAeasy minikit (Qiagen) and evaluated by the use of an 2100 bioanalyzer (Agilent Technologies, CA). RNA-Seq analysis was performed using an Illumina HiSeq 2000 system by the Biopolymers Facility at Harvard Medical School. To eliminate the differences represented by individual embryos, each total RNA sample for RNA-Seq was pooled from three biologically different E14.5 tooth germs with equal amounts of total RNAs. The transcriptomic profiles of the *Yap* CKO and wild-type tooth germs, as well as those of the YAP Tg and wild-type mouse tooth germs, were compared. (Data corresponding to the RNA-Seq Raw sequencing reads and aligned reads are available through the Gene Expression Omnibus.) The RNA-Seq raw data were analyzed using DNAnexus software and further validated using quantitative PCR (qPCR). The qPCR primers used were as follows: for mouse *Yap*, 5′-CCCGACTCCTTCTTCAAGC-3′ and 5′-CTCGAACATGCTGTGGAGTC-3′; for mouse *Hoxa1*, 5′-CTTCTCCAGCGCAGACCTT-3′ and 5′-CTGTGAGCTGCTTGGTGGT-3′; for mouse *Hoxc13*, 5′-GGAAGTCTCCCTTCCCAGAC-3′ and 5′-CTGGCTGCGTACTCCTTCTC-3′; for human *YAP*, 5′-GAACCCCAGATGACTTCCTG-3′ and 5′-CTCCTTCCAGTGTTCCAAGG-3′; for human *HOXA1*, 5′-ACATCTTCTCCAGCGCAGAC-3′ and 5′-CGTGAGCTGCTTGGTAGTGA-3′; and for human *HOXC13*, 5′-TCAGGTGTACTGCTCCAAGG-3′ and 5′-CAGCTGCACCTTAGTGTAGGG-3′.

Immunofluorescence staining and *in situ* hybridization. Immunofluorescence staining and *in situ* hybridization with digoxigenin-labeled RNA probes were conducted on paraffin sections as previously described (26, 29). The antibody against YAP was purchased from Santa Cruz. The antibodies against mouse E-cadherin and P-cadherin were from Life Technologies.

ChIP. The skin samples were collected from the E14.5 *YAP* Tg embryos, fixed in 1% formaldehyde, sonicated, and then immunoprecipitated and collected using YAP or control IgG antibodies (Cell Signaling) according to the manufacturer's protocol (Millipore). Recovered chromatin DNA samples were used for detection of the putative TEAD binding site-containing fragment. Equal amounts of the eluted DNA samples were used for PCR. The chromatin immunoprecipitation (ChIP)-PCR primer pair for moue *Hoxa1* was 5′-CACCACAATCGACCTACAGC-3′ and 5′-CTTTCTTGCAGGCCTCCTGT-3′. The ChIP-PCR primer pair for mouse *Hoxc13* was 5′-ACCCTCTCAGCCAGCCTTA-3′ and 5′-GTCAGCATGGTCGGTCTTC-3′.

Microarray data accession number. RNA-Seq raw sequencing reads and aligned reads are available through the Gene Expression Omnibus at accession no. GSE65524.

RESULTS

Yap deficiency in the dental epithelium results in small tooth size with reduced epithelial cell proliferation. Previous studies showed that *Yap* is expressed in skin progenitor cells and that loss of YAP in the epidermis results in a thin and fragile skin with reduced epithelial progenitor cell proliferation (24, 27). In this study, we analyzed the tooth phenotype of *Yap* conditional knockout mice in which *Yap* was deleted in the oral and dental epithelium as well as in the epidermis of the skin under the control of the human keratin 14 promoter (*K14-Cre*; $Yap^{fl/fl}$ [*Yap* CKO]). *Yap* CKO mice either were aborted or died shortly after birth due to dehydration (27). We examined the tooth phenotype of *Yap* CKO mice at different embryonic stages. At E13.5, *Yap* CKO mice exhibited tooth budding similar to that in control mice (Fig. 1A and B). At E14.5, wild-type tooth germ developed into a cap stage with distinct histodifferentiation of enamel organ and the underlying dental papilla mesenchyme (Fig. 1C). YAP transcripts and proteins were detected in both dental epithelium and mesenchyme, with intense expression in the outer and inner dental epithelial cells (Fig. 1I and K). Conditional knockout of *Yap* under the control of the human keratin 14 promoter resulted in a small tooth germ with reduced expression of YAP transcripts and proteins in the oral and dental epithelial tissues (Fig. 1D, J, and L). However, in the E14.5 *Yap* CKO tooth, we still observed the histodifferentiation of outer and inner dental epithelial cells and enamel knot at the tip of the enamel organ, as well as condensed dental mesenchymal cells underlying the enamel organ (Fig. 1D). At the E16.5 and E18.5 bell stage of tooth development, although *Yap* CKO tooth germs were smaller than those of their control littermates, they exhibited a typical pattern of histodifferentiation, with outer and inner dental epithelia as well as star-shaped stellate reticulum cells in the center of the enamel organ (Fig. 1E to H).

To examine whether the reduced tooth size in the *Yap* CKO mice was due to reduced cell proliferation or to increased cell death, we performed EdU incorporation assay and TUNEL stain-

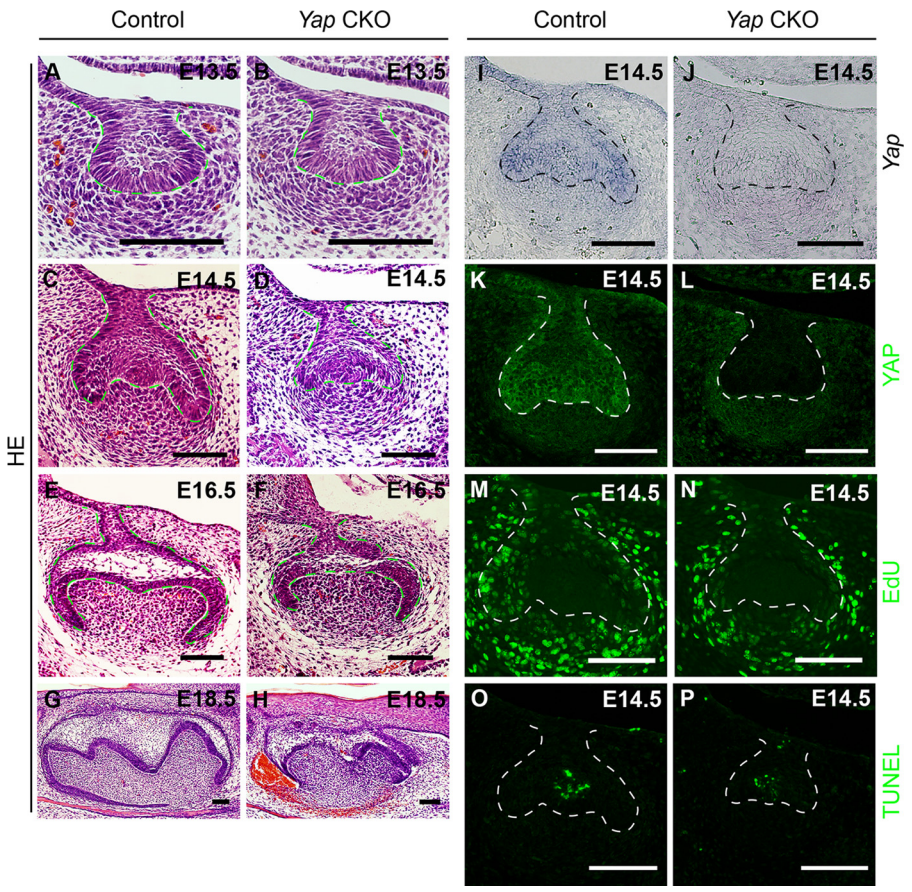

FIG 1 *Yap* deficiency in the dental epithelium leads to development of a small tooth germ with reduced cell proliferation. (A and B) E13.5 *Yap* CKO tooth germ exhibited budding similar to that seen in wild types. (C and D) At E14.5, wild-type tooth developed into a cap stage with inner and outer dental epithelia and a compact cluster of enamel knot cells at the tip of the enamel organ. Condensed dental mesenchymal cells were immediately underneath the enamel organ. The E14.5 *Yap* CKO tooth was smaller than that in wild-type mice but still exhibited histodifferentiation of the enamel organ and condensed dental mesenchyme. (E to H) At the E16.5 and E18.5 bell stage of tooth development, *Yap* CKO tooth germs were smaller than the control tooth germs. However, they still exhibited a typical pattern of histodifferentiation, with outer and inner dental epithelia as well as star-shaped stellate reticulum cells in the center of the enamel organ. (I and J) *In situ* hybridization of E14.5 mice showed that *Yap* transcripts were downregulated in *Yap* CKO dental epithelium compared with that in wild-type tooth. (K and L) Confocal images of YAP immunofluorescence staining showed reduced YAP protein expression in the dental epithelium of *Yap* CKO tooth compared to that in wild-type tooth. (M and N) EdU incorporation assay revealed profound cell proliferation in the enamel organ and dental mesenchyme of E14.5 tooth germ, with the enamel knot area at the tip of the enamel organ devoid of proliferating cells. Dental epithelial cell proliferation was greatly reduced in the E14.5 *Yap* CKO tooth. (O and P) Apoptotic cells were detected within the enamel knot of wild-type and *Yap* CKO tooth germs in similar patterns. Dashed lines indicate the boundary between the dental epithelium and mesenchyme of tooth germs. Scale bars, 100 μm.

ing to detect cell proliferation and apoptosis, respectively. EdU incorporation assay revealed profound cell proliferation in the outer and inner dental epithelia of E14.5 wild-type tooth germ (Fig. 1M). The enamel knot area at the tip of the tooth germ was devoid of dividing cells (30, 31). Dental epithelial cell proliferation was significantly reduced in E14.5 *Yap* CKO tooth germ compared to that in wild-type tooth germ ($P < 0.01$), whereas cell proliferation in the dental mesenchyme was not dramatically affected (Fig. 1M and N). At E14.5, apoptotic cells were detected within the enamel knot area of both wild-type and *Yap* CKO tooth germs (Fig. 1O and P). These data demonstrated that *Yap* deficiency in the dental epithelium resulted in a small tooth size with reduced dental epithelial cell proliferation but might not have affected the histodifferentiation and apoptosis of tooth germs.

***Hoxa1* and *Hoxc13* genes exhibit changes concurrent with those of *Yap* in the *Yap* CKO and *YAP* Tg dental epithelial tissues and skin epidermis.** To decipher the molecular mechanisms underlying the tooth phenotypes of *Yap* CKO mice, we first analyzed the expression patterns of *Shh*, *Fgf4*, *Sox9*, *Notch1*, *Fgf3*, and *Wnt3a* transcripts using *in situ* hybridization and found expression patterns of these genes in E14.5 *Yap* CKO tooth similar to those in the wild types (Fig. 2A to L). *Shh*, *Fgf4*, *Fgf3*, and *Wnt3a* continued to show high levels of expression in the enamel knot area, further indicating that *Yap* deficiency in the dental epithelium may not affect the histodifferentiation of the enamel organ. Previous studies showed that E-cadherin ligation can sequester YAP in the cytoplasm, where it is transcriptionally inert (32). Our recent study showed that overexpression of YAP in the dental epithelium caused dysregulation of E- and P-cadherin proteins in the enamel organ (26). However, we observed expression patterns of E-cadherin and P-cadherin proteins in the E14.5 *Yap* CKO tooth that were similar to those in wild-type tooth (Fig. 2M to P).

To identify the downstream target genes of YAP involved in

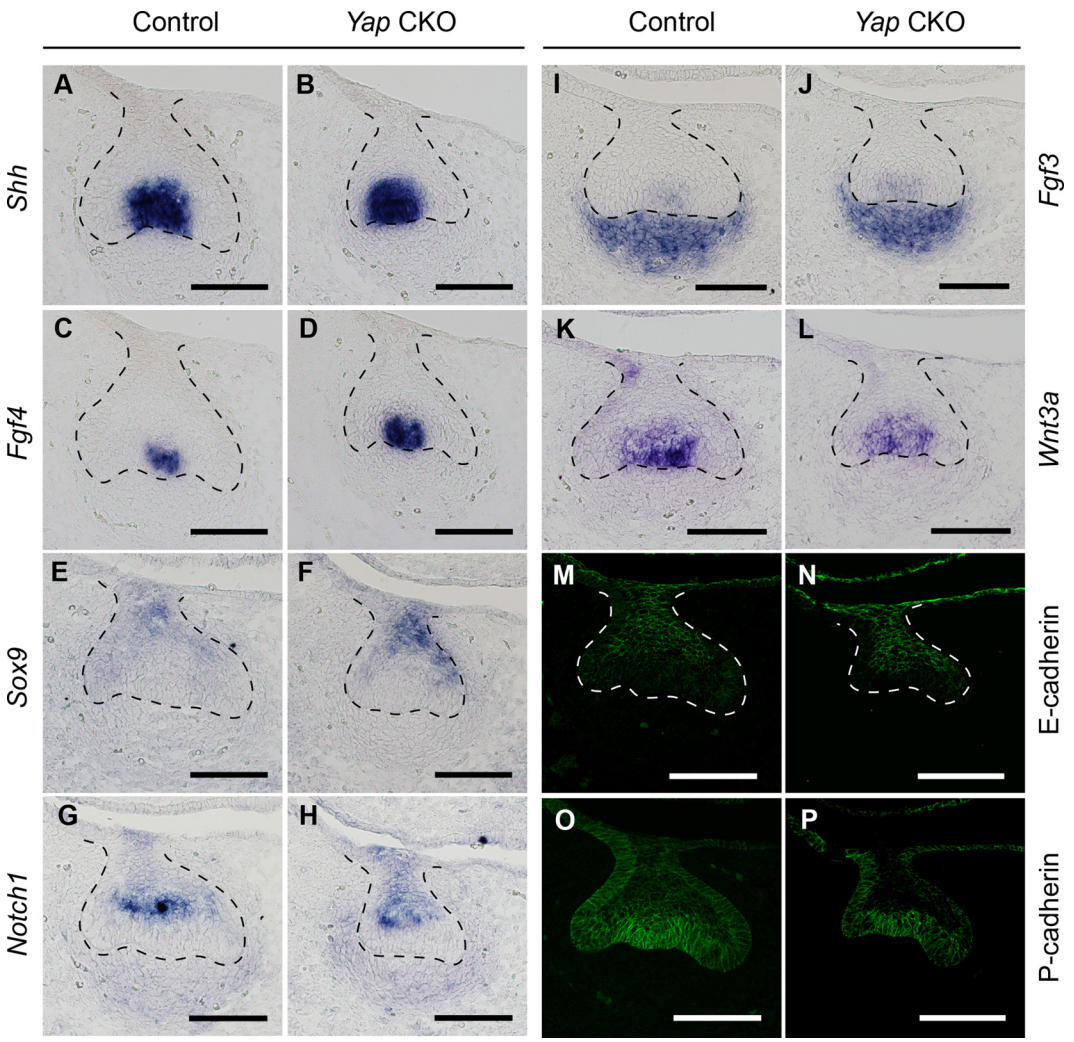

FIG 2 *Yap* deficiency in the dental epithelium does not affect the expression of some key signaling and adhesion molecules. (A to L) At E14.5, the levels of expression of *Shh*, *Fgf4*, *Sox9*, *Notch1*, *Fgf3*, and *Wnt3a* in wild-type and *Yap* CKO tooth germs were similar. *Shh*, *Fgf3*, *Fgf4*, and *Wnt3a* were expressed in the enamel knot area at the tip of developing tooth germs. (M and N) Confocal images of E-cadherin immunofluorescence staining showed similar expression levels of E-cadherin in wild-type and *Yap* CKO tooth germs. (O and P) Confocal images of P-cadherin immunofluorescence staining showed that P-cadherin was highly expressed in the inner and outer dental epithelia and enamel knot cells of the enamel organ. The expression of P-cadherin in the *Yap* CKO tooth was similar to that in wild-type tooth. Dashed lines indicate the boundary between dental epithelium and mesenchyme. Scale bars, 100 μm.

tooth development, we utilized the *Yap* CKO mouse line and also the *YAP* transgenic mice (*YAP* Tg) in which a human *YAP* transgene was inserted and constitutively activated in the mouse genome upon doxycycline (Dox) administration (1). We dissected E14.5 developing tooth germs from both *Yap* CKO and *YAP* Tg mice, extracted their total RNAs, and compared their transcriptional profiles with those of their wild-type littermates by the use of RNA-Seq. The differentially expressed genes identified from the RNA-Seq analysis were further verified using real-time PCR and *in situ* hybridization. Previous studies showed that overexpression of YAP in mouse liver upregulates *Birc2* and *Birc5* transcripts (2). *salv* deficiency in mouse heart tissues causes increased YAP activity and also upregulates the expression of *Birc2* and *Birc5* (20). YAP overexpression increases the expression of *Cyr61* in primary mouse keratinocytes and also activates *AREG* and *CTGF* in human MCF10A mammary epithelial cells (15, 23, 24). In the E14.5 *YAP* Tg mouse tooth germs and skin, *Areg*, *Birc2*, *Birc5*, *Ctgf*, and *Cyr61* showed expression levels similar to those in wild-type mice. In E14.5 *Yap* CKO mice, however, the expression levels of *Ctgf* and *Cyr61* were significantly increased in the skin and dental epithelia (unpublished data). These results further demonstrate that the downstream targets of YAP are regulated in a cell- and tissue-context-dependent manner (10).

Our RNA-Seq data revealed that some *Hox* genes had dramatic changes, with altered *Yap* expression. Interestingly, we found significant changes of *Hoxa1* and *Hoxc13* transcripts concurrent with changes of *Yap* transcripts in both *Yap* CKO and *YAP* Tg mouse tooth germs. In the *Yap* CKO mice, the levels of transcripts of *Yap*, *Hoxa1*, and *Hoxc13* were significantly reduced compared to those in their wild-type littermates (Fig. 3A). In contrast, in the *YAP* Tg mice, the levels of expression of *Yap*, *Hoxa1*, and *Hoxc13* were all significantly upregulated compared to those in their wild-type littermates (Fig. 3B). In addition, however, qPCR results showed that the relative levels of expression of *Hoxa2*, *Hoxa3*, *Hoxa5*,

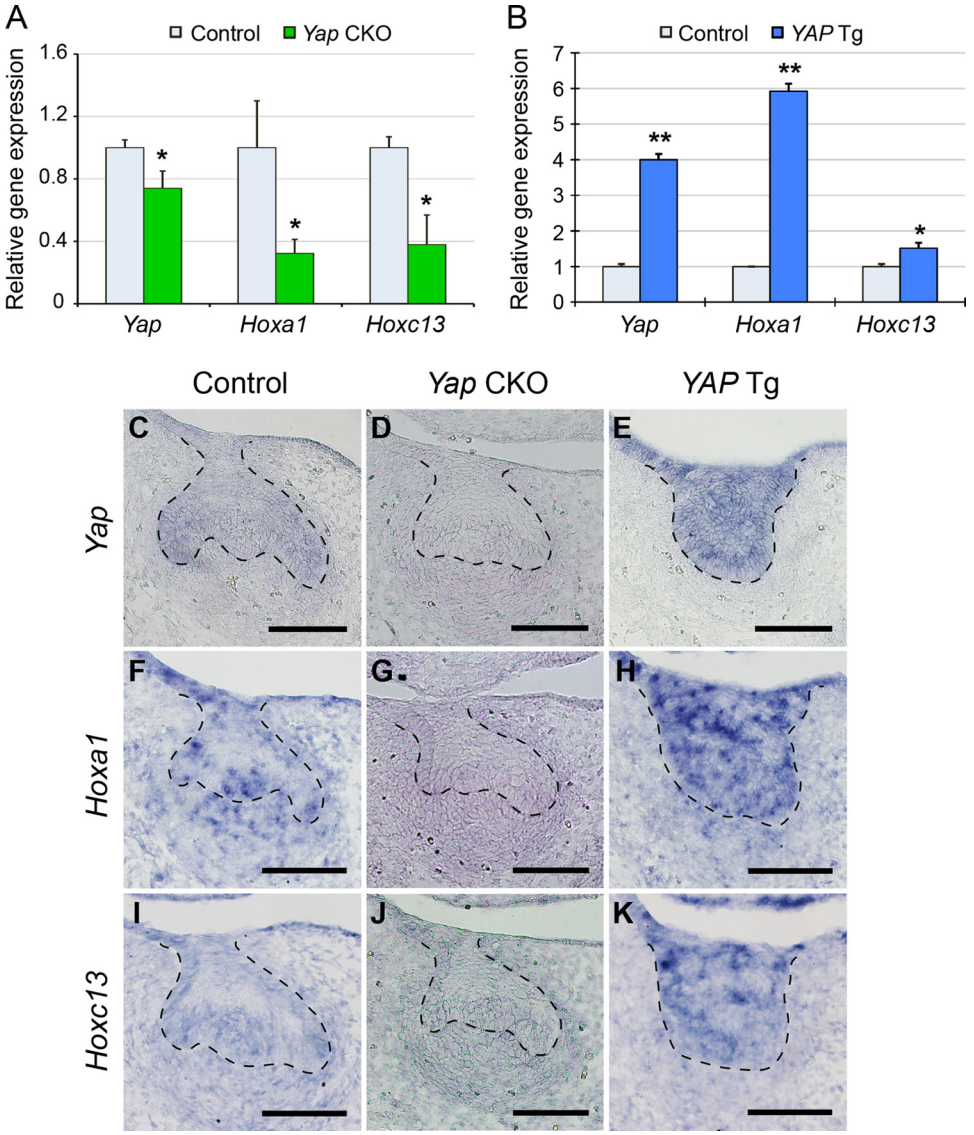

FIG 3 *Hoxa1* and *Hoxc13* transcripts show changes concurrent with those of *Yap* in E14.5 tooth germs. (A) The expression levels of *Hoxa1* and *Hoxc13* were significantly decreased and *Yap* transcript levels were reduced in E14.5 *Yap* CKO tooth germs compared to those in wild types. (B) The expression levels of *Hoxa1* and *Hoxc13* were significantly increased and *Yap* expression levels were elevated in the E14.5 *YAP* Tg tooth germs compared to those in wild-type mice. (C to K) During E14.5 cap-stage tooth development, *Yap* is expressed in both dental epithelial and mesenchymal tissues, with intense expression in the inner and outer dental epithelia. *Hoxa1* and *Hoxc13* showed intense expression in the outer dental epithelium and enamel knot area. *Yap*, *Hoxa1*, and *Hoxc13* mRNA transcripts were all significantly downregulated in the dental epithelial cells of *Yap* CKO mice, whereas they were significantly increased in the *YAP* Tg tooth germs. In panels A and B, data represent the experimental means of the results from three biologically different samples ± standard errors of the means (SEM). *, $P < 0.05$; **, $P < 0.01$. Dashed lines indicate the boundaries between dental epithelium and mesenchyme. Scale bars, 100 μm.

Hoxb9, *Hoxc4*, *Hoxc8*, and *Hoxd1* were not significantly changed in the *Yap* CKO and YAP Tg mouse tooth germs compared to those in wild-type mouse tooth germs (unpublished data), although RNA-Seq results demonstrated differential expression levels of these *Hox* genes. We then performed *in situ* hybridization analysis and found that levels of *Yap* transcripts were greatly reduced in the dental epithelium of E14.5 *Yap* CKO tooth and were greatly increased in the oral and dental epithelium of E14.5 *YAP* Tg tooth (Fig. 3C to E). At E14.5, *Hoxa1* was expressed in both dental epithelial and dental mesenchymal tissues, with a high level of expression in the outer dental epithelium and enamel knot cells (Fig. 3F). High levels of *Hoxc13* transcripts were also detected in the outer and inner dental epithelia (Fig. 3I). Both *Hoxa1* and *Hoxc13* expression levels were greatly reduced in the dental epithelial cells of *Yap* CKO tooth germs and were greatly increased in the dental epithelial cells of *YAP* Tg tooth germs (Fig. 3F to K). The changes of *Hoxa1* and *Hoxc13* transcripts concurrent with *Yap* transcript changes indicated that YAP regulates the expression of *Hoxa1* and *Hoxc13* in developing tooth germs.

Similar concurrent changes of *Hoxa1* and *Hoxc13* transcripts with *Yap* changes were also observed in the E14.5 mouse skin epidermis, with decreased expression of *Yap*, *Hoxa1*, and *Hoxc13* in the *Yap* CKO epidermis and increased expression of these genes in the YAP Tg epidermis (Fig. 4A to I and unpublished data). To

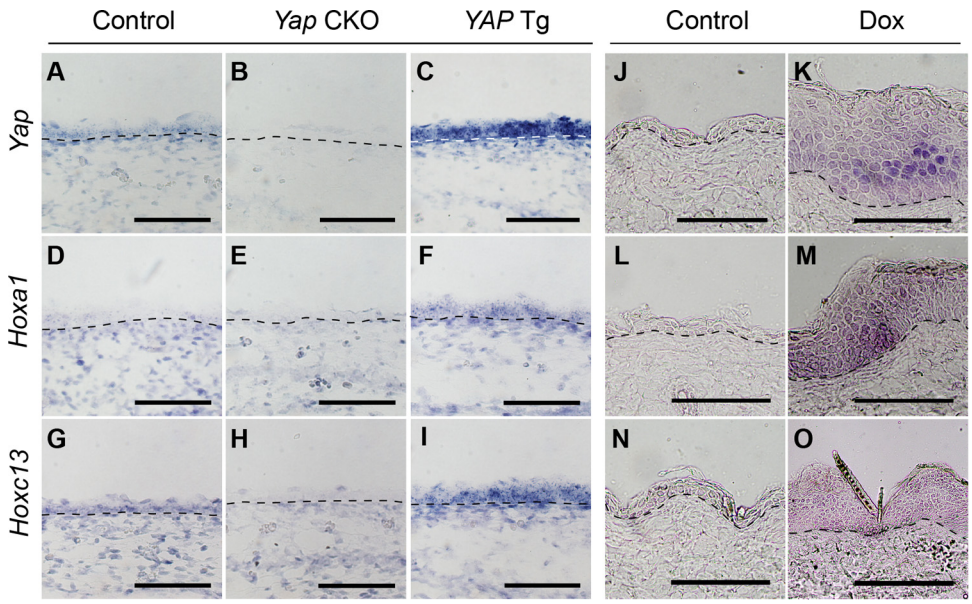

FIG 4 *Hoxa1* and *Hoxc13* transcripts exhibit changes concurrent with those of *Yap* in the epidermis of embryonic and adult mouse skin. (A to I) *Yap*, *Hoxa1*, and *Hoxc13* transcripts were downregulated in E14.5 *Yap* CKO mouse epidermis, whereas they were significantly increased in the *YAP* Tg mouse skin. (J to O) *Yap* mRNA transcripts were upregulated in the epidermis of 10-week-old *YAP* Tg mouse, which was given Dox for 7 days to induce YAP transgene expression. After Dox treatment, the epidermal thickness of *YAP* Tg mouse was significantly increased, and *Hoxa1* and *Hoxc13* mRNA expression levels were also elevated. Dashed lines indicate the boundaries between epidermis and dermis. Scale bars, 100 μm.

study whether YAP also regulates the transcription of *Hoxa1* and *Hoxc13* in adult mice, we activated *YAP* transgene expression in 10-week-old YAP Tg mice by administrating Dox for 7 days. We also observed similar concurrent upregulations of *YAP*, *Hoxa1*, and *Hoxc13* transcripts as well as enhanced cell proliferation in adult mouse epidermis (Fig. 4J to O). These data indicated that YAP regulates the expression of *Hoxa1* and *Hoxc13* in mouse embryonic tooth germs as well as in embryonic and adult mouse epidermis.

YAP regulates the expression of *HOXA1* and *HOXC13* in human keratinocytes. To examine whether YAP also regulates the expression of *HOXA1* and *HOXC13* in human cells, we knocked down *YAP* gene in a human immortalized keratinocyte line, the HaCaT cells (33). When the HaCaT cells were transfected with *YAP* siRNA, levels of *YAP* transcripts were significantly reduced after 24 h, 48 h, and 72 h (Fig. 5A). The relative expression levels of *HOXA1* and *HOXC13* were significantly downregulated after 24 h and 48 h compared with that of scrambled control siRNAs (Fig. 5B and C). Conversely, when we administered Dox in the HaCaT-iYAP^{S127A} cells (27), *YAP* transcripts were significantly upregulated after 6, 24, and 48 h (Fig. 5D). *HOXA1* transcripts were significantly upregulated after 6, 24, and 48 h (Fig. 5E). *HOXC13* transcripts also showed significant immediate upregulation after 6 h (Fig. 5F). These results indicate that YAP regulates *HOXA1* and *HOXC13* expression in human keratinocytes.

The sphere formation assay is an *in vitro* technique to analyze the clonogenic growth potential of progenitor cells or neoplastic cells (13, 34). Previous studies showed that YAP regulates the proliferation of epithelial progenitor cells and is involved in the expansion of progenitor cell pools (1, 13, 27). To examine the role of *HOXA1* and *HOXC13* in YAP activity, we performed sphere formation assay using the HaCaT-iYAP^{S127A} cells. Dox administration in the HaCaT-iYAP^{S127A} cells caused constitutive activation of YAP (27) and significantly increased the numbers of spheres formed from the single cells ($P < 0.01$) (unpublished data). We then transfected YAP siRNA into Dox-administered HaCaT-iYAP^{S127A} cells and found a remarkable reduction of sphere formation compared with those transfected with scrambled control siRNAs ($P < 0.05$) (Fig. 5G), indicating that YAP siRNA efficiently downregulates YAP activity in these cells. Remarkably, when we transfected *HOXA1* or *HOXC13* siRNA into Dox-administered HaCaT-iYAP^{S127A} cells, we also observed a significant reduction of sphere formation ($P < 0.01$) (Fig. 5G). Cotransfection of both *HOXA1* and *HOXC13* siRNAs showed similar reductions of sphere formation in these cells ($P < 0.01$) (Fig. 5G). These results implied that HOXA1 and HOXC13 may be functionally involved in YAP-regulated epithelial progenitor cell proliferation.

Endogenous YAP binds to the *Hoxa1* and *Hoxc13* promoter/enhancer regions containing TEAD binding elements. YAP is a transcriptional coactivator which cannot directly bind to DNA and needs to interact with TEAD transcription factors to regulate its target genes (15, 17, 18, 35–37). YAP is also known to strongly bind to SMAD1, p73, and RUNX2 transcription factors (17, 38–42). We did not find putative SMAD1, p73, and RUNX2 binding elements in the promoter/enhancer regions of *Hoxa1* and *Hoxc13* genes but found several putative TEAD binding elements in mouse and human *Hoxa1* and *Hoxc13* loci (Fig. 6A and B and unpublished data). To examine whether YAP directly regulates *Hoxa1* and *Hoxc13* expression through TEAD transcription factors, we collected E14.5 *YAP* Tg mouse skin samples and performed ChIP assay using YAP antibody. Chromatin isolated from the skin prior to ChIP was used as an input control. The chromatin DNA isolated after the ChIP was used to amplify the corresponding DNA fragments containing the putative TEAD binding sites. PCR amplification using chromatin isolated through the use of normal rabbit IgG antibody served as a negative control. The

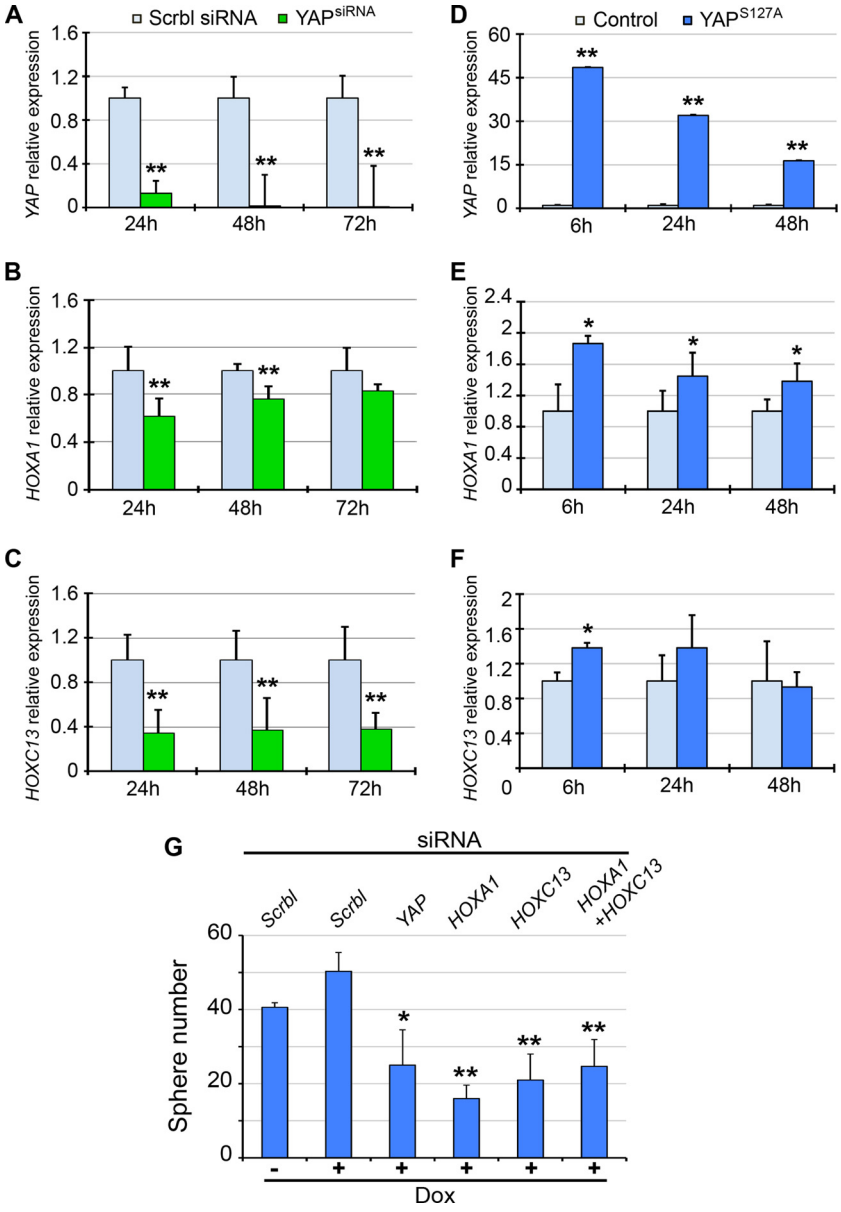

FIG 5 YAP regulates *HOXA1* and *HOXC13* expression in human keratinocytes. (A) *YAP* transcript levels were significantly reduced in HaCaT cells after 24, 48, and 72 h of YAP siRNA treatment. (B) The relative expression levels of *HOXA1* were significantly downregulated in HaCaT cells after 24 h and 48 h of YAP siRNA treatment. (C) The relative expression levels of *HOXC13* were significantly downregulated in HaCaT cells after 24 h and 48 h of YAP siRNA treatment. (D) The expression levels of *YAP* were significantly increased in the HaCaT-iYAP^{S127A} cells at 6 h, 24 h, and 48 h after Dox administration. (E) The relative expression levels of *HOXA1* were significantly upregulated in HaCaT-iYAP^{S127A} cells at 6 h, 24 h, and 48 h after Dox administration. (F) The relative expression levels of human *HOXC13* were significantly upregulated in the HaCaT-iYAP^{S127A} cells at 6 h after Dox administration. (G) Sphere formation assay indicated that the number of spheres formed from single HaCaT-iYAP^{S127A} cells treated with Dox was greatly reduced in YAP siRNA-treated cells compared to those treated with scrambled (Scrbl) control siRNAs ($P < 0.05$). Either *HOXA1* or *HOXC13* siRNA treatment alone or a combination of *HOXA1* siRNA treatment and *HOXC13* siRNA treatment significantly decreased the number of spheres formed from single HaCaT-iYAP^{S127A} cells treated with Dox ($P < 0.01$). *, $P < 0.05$; **, $P < 0.01$. $n = 3$.

eluted chromatin immunoprecipitated by YAP antibody produced a clear 310-bp DNA product containing the putative TEAD binding site (CAGCATCT) in the *Hoxa1* locus and a clear 359-bp band containing two putative TEAD binding sites (TTGTATTT) in the *Hoxc13* locus (Fig. 6A to D). These results suggested that endogenous YAP directly regulates transcriptions of *Hoxa1* and *Hoxc13*, possibly through interaction with TEAD transcription factors (Fig. 6).

DISCUSSION

The Hippo signaling pathway plays critical roles in regulating stem/progenitor cell proliferation, organ size control, and tumorigenesis (1, 2, 4–9). Although the core components of the Hippo pathway are highly conserved between invertebrates and vertebrates, the downstream targets of Hippo/YAP signaling seems to be cell and tissue dependent (10). We previously reported that *Yap* is expressed in both dental epithelial and dental mesenchymal

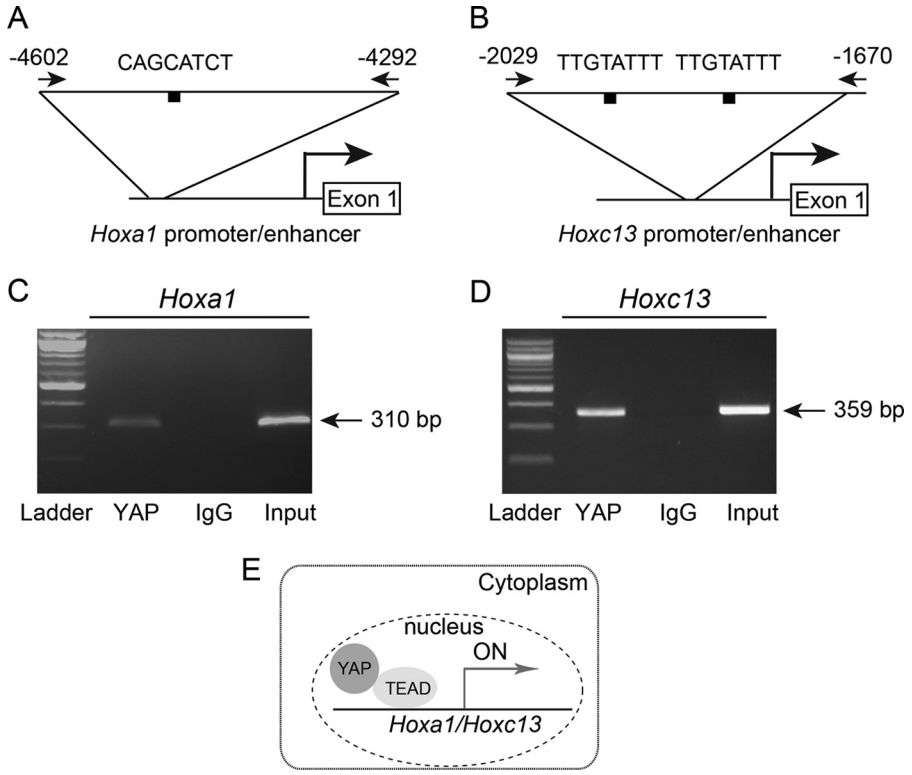

FIG 6 Endogenous YAP binds to the *Hoxa1* and *Hoxc13* promoter/enhancer regions containing TEAD binding elements. (A and B) Schematic representation of the sequence and location of putative TEAD binding sites in mouse *Hoxa1* and *Hoxc13* promoter/enhancer regions. One putative TEAD binding sequence (CAGCATCT) is at −4586 to −4578 in mouse *Hoxa1* (A), and two putative TEAD binding sequences (TTGTATTT) are at −1950 to −1942 and at −1761 to −1753 in mouse *Hoxc13* (B). Arrow pairs indicate the locations of the PCR primers. (C and D) ChIP of endogenous YAP binding to a TEAD binding element within the *Hoxa1* and *Hoxc13* promoter/enhancer regions in E14.5 *YAP* Tg mouse skin. A 310-bp PCR product containing CAGCATCT in the mouse *Hoxa1* gene was amplified from isolated chromatin DNA pulled down through YAP antibody. (D) A 359-bp PCR product containing TTGTATTT in the mouse *Hoxc13* gene was amplified from isolated chromatin DNA pulled down through YAP antibody. The input chromatin is shown as a positive control for the ChIP. (E) A schematic representation of the regulation of *Hoxa1* and *Hoxc13* by YAP through TEAD transcription factors. ON, transcription of *Hoxa1* and *Hoxc13* is active when the YAP-TEAD complex is recruited into the promoter/enhancer regions of *Hoxa1* and *Hoxc13* genes.

tissues of developing tooth and that overexpression of YAP affected tooth morphogenesis and the patterning of enamel knot (26). In the present study, we analyzed the effect of loss of function of YAP in the developing mouse tooth germ and found that *Yap* deficiency in the dental epithelium resulted in a small tooth germ with reduced dental epithelial cell proliferation. *Shh*, *Fgf3*, *Fgf4*, *Sox9*, *Notch1*, and *Wnt3a* transcripts were expressed in a pattern in *Yap* CKO tooth germ similar to that in wild-type tooth, suggesting that loss of function of YAP in the dental epithelium did not affect the histodifferentiation of enamel organ. Some previously reported targets of YAP, such as *Areg*, *Birc2*, *Birc5*, *Ctgf*, and *Cyr61*, did not show altered expression in the *Yap* CKO tooth germs. Interestingly, we identified *Hoxa1* and *Hoxc13* as direct downstream targets of YAP in epithelial tissues of mouse embryonic tooth germs and skin, as well as in adult mouse epidermis and human keratinocytes.

YAP regulates *Hoxa1* and *Hoxc13* expression in the dental epithelium of developing tooth and skin epidermis. *Hox* genes encode a set of evolutionally conserved transcriptional factors which share a 60-amino-acid helix-turn-helix DNA binding homeodomain and define cellular identities along the major and secondary body axis. There are 39 *Hox* genes in mouse and human genomes; those genes are arranged in four linkage groups (Hoxa, Hoxb, Hoxc, and Hoxd) on four separate chromosomes. Based on their sequence similarities and locations within the linkage groups, *Hox* genes are further categorized into 13 paralogous clusters (43). The spatial and temporal expression orders of *Hox* genes along the anterior-to-posterior axis of the embryo are colinear with their chromosomal organizations; the first gene located at the 3′ end of the cluster is transcribed in the more anterior body segments, whereas genes situated more 5′ are progressively expressed in the more posterior areas. Therefore, a particular Hox product defines the morphology of a specific body segment and thus controls the axial patterning of body segments during embryonic patterning (44–46). Mutations of *Hox* genes can cause homeotic transformations and produce new body segments, such as legs growing in place of antenna or an extra set of wings in *Drosophila* (47–49), as well as multiple developmental defects in mice and humans (46).

The regulation of Hox genes is highly complex and involves an intricate combination of local and long-range *cis*-regulatory elements (50–53). Recent studies revealed that polycomb group proteins are critical in silencing *Hox* genes through modulation of chromatin structure. Dynamic patterns of histone marks and higher-order chromatin structure are also important determinants of *Hox* gene regulation (54–58). To date, much progress has been made in defining the *cis*-regulatory elements controlling *Hox* gene expression. In contrast, the nature of the signals and tran-

scription factors regulating *Hox* gene expression remains largely unknown. So far, only a few transcription factors have been identified to regulate *Hox* gene expression. For instance, Krox20 was shown to be required for the induction of *Hoxa2* and *Hoxb2* in rhombomeres 3 and 5, and Kreisler can induce *Hoxa3* and *Hoxb3* expression in rhombomeres 5 and 6 of developing hindbrain (53, 59–61). Cdx proteins have been shown to modulate the expression of multiple *Hox* genes in the more posterior areas of both mesoderm and neural tissues (62, 63). In addition, retinoid acid signal also plays an essential role in regulating the timing of *Hox* gene induction in vertebrates (64). Several retinoic acid response elements (RARE) located in the vicinity of genes *Hox1* to *Hox4* are necessary for the temporal colinear initiation of *Hox* genes (65–68).

By using RNA-Seq analysis, real-time PCR, and *in situ* hybridization in both *Yap* CKO and *YAP* Tg mice, we found changes of *Hoxa1* and *Hoxc13* transcripts concurrent with *Yap* expression changes in dental epithelium of developing tooth and skin epidermis, including downregulation of *Hoxa1* and *Hoxc13* in the *Yap* CKO mice and upregulation of those genes in the *YAP* Tg mice. Same concurrent changes were also observed in the human HaCaT immortalized keratinocytes as well as in adult mouse skin epidermis, suggesting that *Hoxa1* and *Hoxc13* are downstream targets of YAP in these ectoderm-derived tissues.

YAP is a transcriptional coactivator that is brought to its target gene promoters/enhancers by associating with diverse DNA binding transcription factors, such as TEAD, RUNX, p73, or SMAD (15, 17, 18, 35–37). In most tissues, there is at least one TEAD expressed, supporting the idea of a ubiquitous role of YAP-TEAD transcription complex in cell proliferation and survival (21, 35, 69, 70). A screen of a human transcription factor library also identified TEADs as the targets that are most potently activated by YAP (15). Knockdown of TEADs or disruption of YAP-TEAD interaction blunts the regulation of YAP-dependent genes and diminishes the activity of YAP in promoting cell proliferation, oncogenic transformation, and epithelium-mesenchyme transition, suggesting that TEAD is required for YAP-induced target gene expression (15, 71). In the promoter/enhancer regions of mouse and human *Hoxa1* and *Hoxc13* genes, we did not find putative DNA binding sites of RUNX2, p73, or SMAD but identified several putative TEAD binding elements. One copy of a TEAD binding sequence (CAGCATCT) is located at −4737 to −4729 in the promoter/enhancer region of the human *HOXA1* locus (unpublished data) that was similar to that at −4586 to −4578 in mouse *Hoxa1* gene (Fig. 6A). There is also one copy of a TEAD binding sequence (TTGTATTT) located at −1735 to −1727 in the promoter/enhancer region of human *HOXC13* locus (unpublished data) that was similar to those at −1950 to −1942 and at −1761 to −1753 in the mouse *Hoxc13* gene (Fig. 6B). Our ChIP assay further suggested that endogenous YAP binds to the *Hoxa1* and *Hoxc13* promoter/enhancer regions containing TEAD binding elements. Sphere formation assay results implied that *Hoxa1* and *Hoxc13* may be functionally involved in YAP-regulated epithelial progenitor cell proliferation. Based on these data, we propose that YAP directly regulates the expression of *Hoxa1* and *Hoxc13* in epithelial tissues of ectoderm-derived organs, including tooth germ and skin, possibly through TEAD transcription factors. Further studies, such as TEAD ChIP assay and direct-site mutagenesis of the putative TEAD binding sites of *Hoxa1* and *Hoxc13*, are needed to confirm that TEAD transcription factors are indeed involved in the direct regulation of *Hoxa1* and *Hoxc13* by YAP.

In the E14.5 *Yap* CKO tooth germs, we noticed that *Hoxa1* and *Hoxc13* expression levels were reduced more than the *Yap* expression levels compared to the results seen with their control littermates (Fig. 3A). In addition, *Hoxa1* and *Hoxc13* transcripts seemed downregulated in both dental epithelial and dental mesenchymal tissues (Fig. 3F and G and I and J). Since Yap may affect gene expression via different target genes through reciprocal epithelium-mesenchyme interactions, we propose that Yap might directly regulate *Hoxa1* and *Hoxc13* expression in the dental epithelium whereas it might regulate mesenchymal *Hoxa1* and *Hoxc13* expression through epithelial Hoxa1/Hoxc13 or some other Yap target genes.

In the present work, we cannot exclude the possibility that some other *Hox* genes are also regulated by the Hippo/Yap pathway in ectodermally derived tissues. Whether the levels of *Hox* gene expression in these tissues follow cluster regulation patterns similar to those seen in axial patterning or whether they are regulated by a totally different mechanism needs further investigation. Additionally, it would be interesting for future research to examine whether TAZ, a homolog of YAP, also regulates *Hox* genes in oral and skin epithelial tissues.

Implication of YAP, Hoxa1, and Hoxc13 in development and tumorigenesis. YAP plays crucial roles in the maintenance of progenitor cells and organ size control and is also involved in vasculogenesis and nervous system development. *Yap* is expressed from E3.5 to E18.5 in mouse heart and liver and intestinal, neural, and skin progenitor cells (19, 27, 72–78). *Yap* conventional knockout mice exhibited shortened body axis and developmental arrests around E8.5 with severe defects in yolk sac vasculogenesis (19). Conditional knockout *Yap* in skin, heart, and liver impaired progenitor cell proliferation and organ regeneration (27, 74–79). *Yap* deficiency in heart also results in severe vascular abnormalities (75–77, 80). YAP overexpression or defects in the upstream components of Hippo signaling result in enlarged imaginal wing disc in *Drosophila*, as well as in enlarged liver and heart in mice (1, 2, 7, 10, 11, 20, 76, 77, 81–84). In addition, YAP is expressed in neural progenitor cells, and loss of function of YAP in chicken neural tube results in increased cell death and premature neuronal differentiation (79). In this study, *Yap* CKO led to smaller tooth germs in mice and reduced dental epithelial cell proliferation.

Notably, *Hoxa1* has also been associated with organ size control. *Hoxa1* mutant mouse embryos exhibited dramatic reductions in the sizes of rhombomeres 4 and 5 and ear hypoplasia (66, 85, 86). The double mutant of *Hoxa1* and *Hoxb1* exhibited lung hypoplasia (87). HOXA1 A218G polymorphism is closely associated with lower cerebellar volume in healthy humans and with increased head circumference in patients with autism (88–90). A homozygous truncating mutation of *HOXA1* in humans causes severe congenital cardiovascular malformation, craniofacial and inner-ear defects, and brainstem abnormalities (91, 92).

In addition, YAP has also been implicated in maintaining basal epidermal progenitors and regulating hair follicle morphogenesis (24, 27, 93, 94). Overexpression of YAP in the basal epidermis gradually leads to alopecia and, eventually, to hair loss in mice (94). *Hoxc13* was reported to be highly expressed in the tail, limbs, and nails in early embryos (95–97). *Hoxc13* deficiency in mice and humans causes external hair loss and nail defects, whereas overexpression of *Hoxc13* in mice results in ulceration and alopecia

(95–100). Our studies showed that both HOXA1 and HOXC13 play important roles in YAP-regulated epithelial progenitor cell proliferation. Whether *Hoxa1* and *Hoxc13* are involved in YAP-associated developmental disorders needs further investigations.

The Hippo/YAP signaling was initially defined as a tumor suppressor pathway in *Drosophila*, and YAP functions as a proto-oncogene. However, recent studies indicated that both gain of function and loss of function of YAP may cause cancer. YAP overexpression can result in oncogenic transformation, and elevated YAP expression and nuclear localization have been observed in mouse mammary and liver tumors, as well as in multiple types of human cancers, including oral and esophageal squamous cell carcinoma, gastric and liver cancers, colonic and lung adenocarcinoma, ovarian cancers, prostate cancers, and brain tumors (2, 10, 13, 14, 70, 101–105). It has recently been reported that loss of YAP activity potently represses the growth of oncogene-induced mammary tumors and that the YAP inhibitor verteporfin suppresses the growth of human breast cancer cell lines (106). On the other hand, YAP has also been reported to have proapoptotic activity and was previously proposed to exert its tumor suppressor function through potentiating p73-mediated apoptosis (36, 73, 104, 107–111). Low expression of cytoplasmic YAP exists in ductal carcinoma of the breast (104). In addition, low expression of YAP is associated with worse outcome in the human luminal A breast cancer subgroup and invasive breast carcinomas (107, 111). In mice, loss of *Yap* in intestine results in crypt hyperplasia and overgrowth with increased Wnt/R-spondin1 hypersensitivity after whole-body irradiation, and complete loss of YAP is associated with high-grade, stage IV colorectal carcinoma in human patients (73).

Similarly, the involvement of *HOX* genes in tumorigenesis has also drawn increasing attention from researchers (46, 112, 113). Alterations of expression patterns of many *HOX* genes are involved in a large range of tumors, such as lung, breast, and ovarian tumors (113–115). High levels of expression of *HOXA1* and *HOXC13* are found in various human tumors. Overexpressed *HOXA1* was detected in oral squamous cell carcinomas, and its expression is correlated with poor prognosis, probably as a consequence of increasing tumor cell proliferation (116). Forced expression of *HOXA1* in human mammary epithelial cells also causes oncogenic transformation and aggressive tumor formation *in vivo* (117). In contrast, recent studies showed that low expression of *HOXA1* is associated with a poor prognosis of small-cell lung cancer and with lower survival rates (118). In addition, both mRNA levels and protein levels of HOXA1 are significantly correlated with chemotherapy response in human patients. *HOXC13* has also been shown to be strongly and progressively expressed in human melanoma and highly expressed in cells of the MCF-7 breast cancer cell line (119, 120). Our findings that *Hoxa1* and *Hoxc13* are direct downstream targets of YAP in the epithelial tissues of developing tooth and the epidermis of skin may provide insights into understanding the molecular mechanisms of YAP in not only organism development but also human disease onset and progression.

ACKNOWLEDGMENTS

We thank Fernando Camargo (Boston Children's Hospital) for kindly providing the *YAP* transgenic and *Yap* CKO mice and the HaCaT-iYAP^{S127A} cells.

The HSDM Dean's Scholar Award supported this investigation.

We declare that we have no potential conflicts of interest with respect to the authorship and/or publication of this article.

REFERENCES

1. **Camargo FD, Gokhale S, Johnnidis JB, Fu D, Bell GW, Jaenisch R, Brummelkamp TR.** 2007. YAP1 increases organ size and expands undifferentiated progenitor cells. Curr Biol **17:**2054–2060. http://dx.doi.org/10.1016/j.cub.2007.10.039.
2. **Dong J, Feldmann G, Huang J, Wu S, Zhang N, Comerford SA, Gayyed MF, Anders RA, Maitra A, Pan D.** 2007. Elucidation of a universal size-control mechanism in Drosophila and mammals. Cell **130:**1120–1133. http://dx.doi.org/10.1016/j.cell.2007.07.019.
3. **Grusche FA, Degoutin JL, Richardson HE, Harvey KF.** 2011. The Salvador/Warts/Hippo pathway controls regenerative tissue growth in Drosophila melanogaster. Dev Biol **350:**255–266. http://dx.doi.org/10.1016/j.ydbio.2010.11.020.
4. **Harvey KF, Pfleger CM, Hariharan IK.** 2003. The Drosophila Mst ortholog, hippo, restricts growth and cell proliferation and promotes apoptosis. Cell **114:**457–467. http://dx.doi.org/10.1016/S0092-8674(03)00557-9.
5. **Jia J, Zhang W, Wang B, Trinko R, Jiang J.** 2003. The Drosophila Ste20 family kinase dMST functions as a tumor suppressor by restricting cell proliferation and promoting apoptosis. Genes Dev **17:**2514–2519. http://dx.doi.org/10.1101/gad.1134003.
6. **Pantalacci S, Tapon N, Leopold P.** 2003. The Salvador partner Hippo promotes apoptosis and cell-cycle exit in Drosophila. Nat Cell Biol **5:**921–927. http://dx.doi.org/10.1038/ncb1051.
7. **Udan RS, Kango-Singh M, Nolo R, Tao C, Halder G.** 2003. Hippo promotes proliferation arrest and apoptosis in the Salvador/Warts pathway. Nat Cell Biol **5:**914–920. http://dx.doi.org/10.1038/ncb1050.
8. **Wu S, Huang J, Dong J, Pan D.** 2003. hippo encodes a Ste-20 family protein kinase that restricts cell proliferation and promotes apoptosis in conjunction with salvador and warts. Cell **114:**445–456. http://dx.doi.org/10.1016/S0092-8674(03)00549-X.
9. **Zhao B, Wei X, Li W, Udan RS, Yang Q, Kim J, Xie J, Ikenoue T, Yu J, Li L, Zheng P, Ye K, Chinnaiyan A, Halder G, Lai ZC, Guan KL.** 2007. Inactivation of YAP oncoprotein by the Hippo pathway is involved in cell contact inhibition and tissue growth control. Genes Dev **21:**2747–2761. http://dx.doi.org/10.1101/gad.1602907.
10. **Pan D.** 2010. The hippo signaling pathway in development and cancer. Dev Cell **19:**491–505. http://dx.doi.org/10.1016/j.devcel.2010.09.011.
11. **Huang J, Wu S, Barrera J, Matthews K, Pan D.** 2005. The Hippo signaling pathway coordinately regulates cell proliferation and apoptosis by inactivating Yorkie, the Drosophila homolog of YAP. Cell **122:**421–434. http://dx.doi.org/10.1016/j.cell.2005.06.007.
12. **Zhang X, George J, Deb S, Degoutin JL, Takano EA, Fox SB, Bowtell DD, Harvey KF.** 2011. The Hippo pathway transcriptional co-activator, YAP, is an ovarian cancer oncogene. Oncogene **30:**2810–2822. http://dx.doi.org/10.1038/onc.2011.8.
13. **Overholtzer M, Zhang J, Smolen GA, Muir B, Li W, Sgroi DC, Deng CX, Brugge JS, Haber DA.** 2006. Transforming properties of YAP, a candidate oncogene on the chromosome 11q22 amplicon. Proc Natl Acad Sci U S A **103:**12405–12410. http://dx.doi.org/10.1073/pnas.0605579103.
14. **Zender L, Spector MS, Xue W, Flemming P, Cordon-Cardo C, Silke J, Fan ST, Luk JM, Wigler M, Hannon GJ, Mu D, Lucito R, Powers S, Lowe SW.** 2006. Identification and validation of oncogenes in liver cancer using an integrative oncogenomic approach. Cell **125:**1253–1267. http://dx.doi.org/10.1016/j.cell.2006.05.030.
15. **Zhao B, Ye X, Yu J, Li L, Li W, Li S, Lin JD, Wang CY, Chinnaiyan AM, Lai ZC, Guan KL.** 2008. TEAD mediates YAP-dependent gene induction and growth control. Genes Dev **22:**1962–1971. http://dx.doi.org/10.1101/gad.1664408.
16. **Zhao B, Lei QY, Guan KL.** 2008. The Hippo-YAP pathway: new connections between regulation of organ size and cancer. Curr Opin Cell Biol **20:**638–646. http://dx.doi.org/10.1016/j.ceb.2008.10.001.
17. **Lapi E, Di Agostino S, Donzelli S, Gal H, Domany E, Rechavi G, Pandolfi PP, Givol D, Strano S, Lu X, Blandino G.** 2008. PML, YAP, and p73 are components of a proapoptotic autoregulatory feedback loop. Mol Cell **32:**803–814. http://dx.doi.org/10.1016/j.molcel.2008.11.019.
18. **Fujii M, Toyoda T, Nakanishi H, Yatabe Y, Sato A, Matsudaira Y, Ito H, Murakami H, Kondo Y, Kondo E, Hida T, Tsujimura T, Osada H,**

Sekido Y. 2012. TGF-beta synergizes with defects in the Hippo pathway to stimulate human malignant mesothelioma growth. J Exp Med **209:** 479–494. http://dx.doi.org/10.1084/jem.20111653.
19. **Morin-Kensicki EM, Boone BN, Howell M, Stonebraker JR, Teed J, Alb JG, Magnuson TR, O'Neal W, Milgram SL.** 2006. Defects in yolk sac vasculogenesis, chorioallantoic fusion, and embryonic axis elongation in mice with targeted disruption of Yap65. Mol Cell Biol **26:**77–87. http://dx.doi.org/10.1128/MCB.26.1.77-87.2006.
20. **Heallen T, Zhang M, Wang J, Bonilla-Claudio M, Klysik E, Johnson RL, Martin JF.** 2011. Hippo pathway inhibits Wnt signaling to restrain cardiomyocyte proliferation and heart size. Science **332:**458–461. http://dx.doi.org/10.1126/science.1199010.
21. **Lian I, Kim J, Okazawa H, Zhao J, Zhao B, Yu J, Chinnaiyan A, Israel MA, Goldstein LS, Abujarour R, Ding S, Guan KL.** 2010. The role of YAP transcription coactivator in regulating stem cell self-renewal and differentiation. Genes Dev **24:**1106–1118. http://dx.doi.org/10.1101/gad.1903310.
22. **Thompson BJ, Cohen SM.** 2006. The Hippo pathway regulates the bantam microRNA to control cell proliferation and apoptosis in Drosophila. Cell **126:**767–774. http://dx.doi.org/10.1016/j.cell.2006.07.013.
23. **Zhang J, Ji JY, Yu M, Overholtzer M, Smolen GA, Wang R, Brugge JS, Dyson NJ, Haber DA.** 2009. YAP-dependent induction of amphiregulin identifies a non-cell-autonomous component of the Hippo pathway. Nat Cell Biol **11:**1444–1450. http://dx.doi.org/10.1038/ncb1993.
24. **Zhang H, Pasolli HA, Fuchs E.** 2011. Yes-associated protein (YAP) transcriptional coactivator functions in balancing growth and differentiation in skin. Proc Natl Acad Sci U S A **108:**2270–2275. http://dx.doi.org/10.1073/pnas.1019603108.
25. **Pispa J, Thesleff I.** 2003. Mechanisms of ectodermal organogenesis. Dev Biol **262:**195–205. http://dx.doi.org/10.1016/S0012-1606(03)00325-7.
26. **Liu M, Zhao S, Wang XP.** 28 February 2014, posting date. YAP overexpression affects tooth morphogenesis and enamel knot patterning. J Dent Res http://dx.doi.org/10.1177/0022034514525784.
27. **Schlegelmilch K, Mohseni M, Kirak O, Pruszak J, Rodriguez JR, Zhou D, Kreger BT, Vasioukhin V, Avruch J, Brummelkamp TR, Camargo FD.** 2011. Yap1 acts downstream of alpha-catenin to control epidermal proliferation. Cell **144:**782–795. http://dx.doi.org/10.1016/j.cell.2011.02.031.
28. **Nguyen H, Rendl M, Fuchs E.** 2006. Tcf3 governs stem cell features and represses cell fate determination in skin. Cell **127:**171–183. http://dx.doi.org/10.1016/j.cell.2006.07.036.
29. **Wang XP, O'Connell DJ, Lund JJ, Saadi I, Kuraguchi M, Turbe-Doan A, Cavallesco R, Kim H, Park PJ, Harada H, Kucherlapati R, Maas RL.** 2009. Apc inhibition of Wnt signaling regulates supernumerary tooth formation during embryogenesis and throughout adulthood. Development **136:**1939–1949. http://dx.doi.org/10.1242/dev.033803.
30. **Vaahtokari A, Aberg T, Jernvall J, Keranen S, Thesleff I.** 1996. The enamel knot as a signaling center in the developing mouse tooth. Mech Dev **54:**39–43. http://dx.doi.org/10.1016/0925-4773(95)00459-9.
31. **Vaahtokari A, Aberg T, Thesleff I.** 1996. Apoptosis in the developing tooth: association with an embryonic signaling center and suppression by EGF and FGF-4. Development **122:**121–129.
32. **Kim NG, Koh E, Chen X, Gumbiner BM.** 2011. E-cadherin mediates contact inhibition of proliferation through Hippo signaling-pathway components. Proc Natl Acad Sci U S A **108:**11930–11935. http://dx.doi.org/10.1073/pnas.1103345108.
33. **Boukamp P, Petrussevska RT, Breitkreutz D, Hornung J, Markham A, Fusenig NE.** 1988. Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line. J Cell Biol **106:**761–771. http://dx.doi.org/10.1083/jcb.106.3.761.
34. **Pastrana E, Silva-Vargas V, Doetsch F.** 2011. Eyes wide open: a critical review of sphere-formation as an assay for stem cells. Cell Stem Cell **8:**486–498. http://dx.doi.org/10.1016/j.stem.2011.04.007.
35. **Vassilev A, Kaneko KJ, Shu H, Zhao Y, DePamphilis ML.** 2001. TEAD/TEF transcription factors utilize the activation domain of YAP65, a Src/Yes-associated protein localized in the cytoplasm. Genes Dev **15:**1229–1241. http://dx.doi.org/10.1101/gad.888601.
36. **Strano S, Munarriz E, Rossi M, Castagnoli L, Shaul Y, Sacchi A, Oren M, Sudol M, Cesareni G, Blandino G.** 2001. Physical interaction with Yes-associated protein enhances p73 transcriptional activity. J Biol Chem **276:**15164–15173. http://dx.doi.org/10.1074/jbc.M010484200.
37. **Yagi R, Chen LF, Shigesada K, Murakami Y, Ito Y.** 1999. A WW domain-containing yes-associated protein (YAP) is a novel transcriptional co-activator. EMBO J **18:**2551–2562. http://dx.doi.org/10.1093/emboj/18.9.2551.
38. **Osada M, Park HL, Nagakawa Y, Begum S, Yamashita K, Wu G, Kim MS, Trink B, Sidransky D.** 2006. A novel response element confers p63- and p73-specific activation of the WNT4 promoter. Biochem Biophys Res Commun **339:**1120–1128. http://dx.doi.org/10.1016/j.bbrc.2005.11.118.
39. **Ethayathulla AS, Tse PW, Monti P, Nguyen S, Inga A, Fronza G, Viadiu H.** 2012. Structure of p73 DNA-binding domain tetramer modulates p73 transactivation. Proc Natl Acad Sci U S A **109:**6066–6071. http://dx.doi.org/10.1073/pnas.1115463109.
40. **Jiménez MJ, Balbín M, López JM, Alvarez J, Komori T, López-Otín C.** 1999. Collagenase 3 is a target of Cbfa1, a transcription factor of the runt gene family involved in bone formation. Mol Cell Biol **19:**4431–4442.
41. **Ducy P, Zhang R, Geoffroy V, Ridall AL, Karsenty G.** 1997. Osf2/Cbfa1: a transcriptional activator of osteoblast differentiation. Cell **89:**747–754. http://dx.doi.org/10.1016/S0092-8674(00)80257-3.
42. **Holmes A, Abraham DJ, Sa S, Shiwen X, Black CM, Leask A.** 2001. CTGF and SMADs, maintenance of scleroderma phenotype is independent of SMAD signaling. J Biol Chem **276:**10594–10601. http://dx.doi.org/10.1074/jbc.M010149200.
43. **Scott MP.** 1992. Vertebrate homeobox gene nomenclature. Cell **71:**551–553. http://dx.doi.org/10.1016/0092-8674(92)90588-4.
44. **Duboule D, Morata G.** 1994. Colinearity and functional hierarchy among genes of the homeotic complexes. Trends Genet **10:**358–364. http://dx.doi.org/10.1016/0168-9525(94)90132-5.
45. **Garcia-Fernàndez J.** 2005. The genesis and evolution of homeobox gene clusters. Nat Rev Genet **6:**881–892. http://dx.doi.org/10.1038/nrg1723.
46. **Quinonez SC, Innis JW.** 2014. Human HOX gene disorders. Mol Genet Metab **111:**4–15. http://dx.doi.org/10.1016/j.ymgme.2013.10.012.
47. **Gardner EJ, Woolf CM.** 1949. Maternal effect involved in the inheritance of abnormal growths in the head region of Drosophila melanogaster. Genetics **34:**573–585.
48. **Struhl G.** 1981. A homoeotic mutation transforming leg to antenna in Drosophila. Nature **292:**635–638. http://dx.doi.org/10.1038/292635a0.
49. **Lewis EB.** 1978. A gene complex controlling segmentation in Drosophila. Nature **276:**565–570. http://dx.doi.org/10.1038/276565a0.
50. **Soshnikova N.** 2014. Hox genes regulation in vertebrates. Dev Dyn **243:**49–58. http://dx.doi.org/10.1002/dvdy.24014.
51. **Sharpe J, Nonchev S, Gould A, Whiting J, Krumlauf R.** 1998. Selectivity, sharing and competitive interactions in the regulation of Hoxb genes. EMBO J **17:**1788–1798. http://dx.doi.org/10.1093/emboj/17.6.1788.
52. **Spitz F, Gonzalez F, Peichel C, Vogt TF, Duboule D, Zakany J.** 2001. Large scale transgenic and cluster deletion analysis of the HoxD complex separate an ancestral regulatory module from evolutionary innovations. Genes Dev **15:**2209–2214. http://dx.doi.org/10.1101/gad.205701.
53. **Sham MH, Vesque C, Nonchev S, Marshall H, Frain M, Gupta RD, Whiting J, Wilkinson D, Charnay P, Krumlauf R.** 1993. The zinc finger gene Krox20 regulates HoxB2 (Hox2.8) during hindbrain segmentation. Cell **72:**183–196. http://dx.doi.org/10.1016/0092-8674(93)90659-E.
54. **Noordermeer D, Leleu M, Splinter E, Rougemont J, De Laat W, Duboule D.** 2011. The dynamic architecture of Hox gene clusters. Science **334:**222–225. http://dx.doi.org/10.1126/science.1207194.
55. **Bracken AP, Dietrich N, Pasini D, Hansen KH, Helin K.** 2006. Genome-wide mapping of Polycomb target genes unravels their roles in cell fate transitions. Genes Dev **20:**1123–1136. http://dx.doi.org/10.1101/gad.381706.
56. **Boyer LA, Plath K, Zeitlinger J, Brambrink T, Medeiros LA, Lee TI, Levine SS, Wernig M, Tajonar A, Ray MK, Bell GW, Otte AP, Vidal M, Gifford DK, Young RA, Jaenisch R.** 2006. Polycomb complexes repress developmental regulators in murine embryonic stem cells. Nature **441:**349–353. http://dx.doi.org/10.1038/nature04733.
57. **Lee TI, Jenner RG, Boyer LA, Guenther MG, Levine SS, Kumar RM, Chevalier B, Johnstone SE, Cole MF, Isono K, Koseki H, Fuchikami T, Abe K, Murray HL, Zucker JP, Yuan B, Bell GW, Herbolsheimer E, Hannett NM, Sun K, Odom DT, Otte AP, Volkert TL, Bartel DP, Melton DA, Gifford DK, Jaenisch R, Young RA.** 2006. Control of developmental regulators by Polycomb in human embryonic stem cells. Cell **125:**301–313. http://dx.doi.org/10.1016/j.cell.2006.02.043.
58. **Portoso M, Cavalli G.** 2008. The role of RNAi and noncoding RNAs in polycomb mediated control of gene expression and genomic programming, p 29–40. *In* Morris KV (ed), RNA and the regulation of gene

expression: a hidden layer of complexity. Caister Academic Press, Norwich, United Kingdom.
59. **Manzanares M, Cordes S, Ariza-McNaughton L, Sadl V, Maruthainar K, Barsh G, Krumlauf R.** 1999. Conserved and distinct roles of kreisler in regulation of the paralogous Hoxa3 and Hoxb3 genes. Development **126:**759–769.
60. **Manzanares M, Nardelli J, Gilardi-Hebenstreit P, Marshall H, Giudicelli F, Martinez-Pastor MT, Krumlauf R, Charnay P.** 2002. Krox20 and kreisler co-operate in the transcriptional control of segmental expression of Hoxb3 in the developing hindbrain. EMBO J **21:**365–376. http://dx.doi.org/10.1093/emboj/21.3.365.
61. **Nonchev S, Maconochie M, Vesque C, Aparicio S, Ariza-McNaughton L, Manzanares M, Maruthainar K, Kuroiwa A, Brenner S, Charnay P, Krumlauf R.** 1996. The conserved role of Krox-20 in directing Hox gene expression during vertebrate hindbrain segmentation. Proc Natl Acad Sci U S A **93:**9339–9345. http://dx.doi.org/10.1073/pnas.93.18.9339.
62. **Subramanian V, Meyer BI, Gruss P.** 1995. Disruption of the murine homeobox gene Cdx1 affects axial skeletal identities by altering the mesodermal expression domains of Hox genes. Cell **83:**641–653. http://dx.doi.org/10.1016/0092-8674(95)90104-3.
63. **van den Akker E, Forlani S, Chawengsaksophak K, de Graaff W, Beck F, Meyer BI, Deschamps J.** 2002. Cdx1 and Cdx2 have overlapping functions in anteroposterior patterning and posterior axis elongation. Development **129:**2181–2193.
64. **Kessel M, Gruss P.** 1991. Homeotic transformations of murine vertebrae and concomitant alteration of Hox codes induced by retinoic acid. Cell **67:**89–104. http://dx.doi.org/10.1016/0092-8674(91)90574-I.
65. **Rhinn M, Dolle P.** 2012. Retinoic acid signalling during development. Development **139:**843–858. http://dx.doi.org/10.1242/dev.065938.
66. **Gavalas A, Studer M, Lumsden A, Rijli FM, Krumlauf R, Chambon P.** 1998. Hoxa1 and Hoxb1 synergize in patterning the hindbrain, cranial nerves and second pharyngeal arch. Development **125:**1123–1136.
67. **Packer AI, Crotty DA, Elwell VA, Wolgemuth DJ.** 1998. Expression of the murine Hoxa4 gene requires both autoregulation and a conserved retinoic acid response element. Development **125:**1991–1998.
68. **Zhang F, Nagy Kovacs E, Featherstone MS.** 2000. Murine hoxd4 expression in the CNS requires multiple elements including a retinoic acid response element. Mech Dev **96:**79–89. http://dx.doi.org/10.1016/S0925-4773(00)00377-4.
69. **Kaneko KJ, Cullinan EB, Latham KE, DePamphilis ML.** 1997. Transcription factor mTEAD-2 is selectively expressed at the beginning of zygotic gene expression in the mouse. Development **124:**1963–1973.
70. **Zhao B, Li L, Lei Q, Guan KL.** 2010. The Hippo-YAP pathway in organ size control and tumorigenesis: an updated version. Genes Dev **24:**862–874. http://dx.doi.org/10.1101/gad.1909210.
71. **Goulev Y, Fauny JD, Gonzalez-Marti B, Flagiello D, Silber J, Zider A.** 2008. SCALLOPED interacts with YORKIE, the nuclear effector of the hippo tumor-suppressor pathway in Drosophila. Curr Biol **18:**435–441. http://dx.doi.org/10.1016/j.cub.2008.02.034.
72. **Ramos A, Camargo FD.** 2012. The Hippo signaling pathway and stem cell biology. Trends Cell Biol **22:**339–346. http://dx.doi.org/10.1016/j.tcb.2012.04.006.
73. **Barry ER, Morikawa T, Butler BL, Shrestha K, de la Rosa R, Yan KS, Fuchs CS, Magness ST, Smits R, Ogino S, Kuo CJ, Camargo FD.** 2013. Restriction of intestinal stem cell expansion and the regenerative response by YAP. Nature **493:**106–110.
74. **Cai J, Zhang N, Zheng Y, de Wilde RF, Maitra A, Pan D.** 2010. The Hippo signaling pathway restricts the oncogenic potential of an intestinal regeneration program. Genes Dev **24:**2383–2388. http://dx.doi.org/10.1101/gad.1978810.
75. **von Gise A, Lin Z, Schlegelmilch K, Honor LB, Pan GM, Buck JN, Ma Q, Ishiwata T, Zhou B, Camargo FD, Pu WT.** 2012. YAP1, the nuclear target of Hippo signaling, stimulates heart growth through cardiomyocyte proliferation but not hypertrophy. Proc Natl Acad Sci U S A **109:**2394–2399. http://dx.doi.org/10.1073/pnas.1116136109.
76. **Xin M, Kim Y, Sutherland LB, Murakami M, Qi X, McAnally J, Porrello ER, Mahmoud AI, Tan W, Shelton JM, Richardson JA, Sadek HA, Bassel-Duby R, Olson EN.** 2013. Hippo pathway effector Yap promotes cardiac regeneration. Proc Natl Acad Sci U S A **110:**13839–13844. http://dx.doi.org/10.1073/pnas.1313192110.
77. **Xin M, Kim Y, Sutherland LB, Qi X, McAnally J, Schwartz RJ, Richardson JA, Bassel-Duby R, Olson EN.** 2011. Regulation of insulin-like growth factor signaling by Yap governs cardiomyocyte proliferation and embryonic heart size. Sci Signal **4:**ra70.
78. **Zhang N, Bai H, David KK, Dong J, Zheng Y, Cai J, Giovannini M, Liu P, Anders RA, Pan D.** 2010. The Merlin/NF2 tumor suppressor functions through the YAP oncoprotein to regulate tissue homeostasis in mammals. Dev Cell **19:**27–38. http://dx.doi.org/10.1016/j.devcel.2010.06.015.
79. **Cao X, Pfaff SL, Gage FH.** 2008. YAP regulates neural progenitor cell number via the TEA domain transcription factor. Genes Dev **22:**3320–3334. http://dx.doi.org/10.1101/gad.1726608.
80. **Wang Y, Hu G, Liu F, Wang X, Wu M, Schwarz JJ, Zhou J.** 2014. Deletion of yes-associated protein (YAP) specifically in cardiac and vascular smooth muscle cells reveals a crucial role for YAP in mouse cardiovascular development. Circ Res **114:**957–965. http://dx.doi.org/10.1161/CIRCRESAHA.114.303411.
81. **Lu L, Li Y, Kim SM, Bossuyt W, Liu P, Qiu Q, Wang Y, Halder G, Finegold MJ, Lee JS, Johnson RL.** 2010. Hippo signaling is a potent in vivo growth and tumor suppressor pathway in the mammalian liver. Proc Natl Acad Sci U S A **107:**1437–1442. http://dx.doi.org/10.1073/pnas.0911427107.
82. **Song H, Mak KK, Topol L, Yun K, Hu J, Garrett L, Chen Y, Park O, Chang J, Simpson RM, Wang CY, Gao B, Jiang J, Yang Y.** 2010. Mammalian MstI and Mst2 kinases play essential roles in organ size control and tumor suppression. Proc Natl Acad Sci U S A **107:**1431–1436. http://dx.doi.org/10.1073/pnas.0911409107.
83. **Lee KP, Lee JH, Kim TS, Kim TH, Park HD, Byun JS, Kim MC, Jeong WI, Calvisi DF, Kim JM, Lim DS.** 2010. The Hippo-Salvador pathway restrains hepatic oval cell proliferation, liver size, and liver tumorigenesis. Proc Natl Acad Sci U S A **107:**8248–8253. http://dx.doi.org/10.1073/pnas.0912203107.
84. **Benhamouche S, Curto M, Saotome I, Gladden AB, Liu CH, Giovannini M, McClatchey AI.** 2010. Nf2/Merlin controls progenitor homeostasis and tumorigenesis in the liver. Genes Dev **24:**1718–1730. http://dx.doi.org/10.1101/gad.1938710.
85. **Lufkin T, Dierich A, LeMeur M, Mark M, Chambon P.** 1991. Disruption of the Hox-1.6 homeobox gene results in defects in a region corresponding to its rostral domain of expression. Cell **66:**1105–1119. http://dx.doi.org/10.1016/0092-8674(91)90034-V.
86. **Mark M, Lufkin T, Vonesch JL, Ruberte E, Olivo JC, Dolle P, Gorry P, Lumsden A, Chambon P.** 1993. Two rhombomeres are altered in Hoxa-1 mutant mice. Development **119:**319–338.
87. **Rossel M, Capecchi MR.** 1999. Mice mutant for both Hoxa1 and Hoxb1 show extensive remodeling of the hindbrain and defects in craniofacial development. Development **126:**5027–5040.
88. **Canu E, Boccardi M, Ghidoni R, Benussi L, Duchesne S, Testa C, Binetti G, Frisoni GB.** 2009. HOXA1 A218G polymorphism is associated with smaller cerebellar volume in healthy humans. J Neuroimaging **19:**353–358. http://dx.doi.org/10.1111/j.1552-6569.2008.00326.x.
89. **Conciatori M, Stodgell CJ, Hyman SL, O'Bara M, Militerni R, Bravaccio C, Trillo S, Montecchi F, Schneider C, Melmed R, Elia M, Crawford L, Spence SJ, Muscarella L, Guarnieri V, D'Agruma L, Quattrone A, Zelante L, Rabinowitz D, Pascucci T, Puglisi-Allegra S, Reichelt KL, Rodier PM, Persico AM.** 2004. Association between the HOXA1 A218G polymorphism and increased head circumference in patients with autism. Biol Psychiatry **55:**413–419. http://dx.doi.org/10.1016/j.biopsych.2003.10.005.
90. **Muscarella LA, Guarnieri V, Sacco R, Militerni R, Bravaccio C, Trillo S, Schneider C, Melmed R, Elia M, Mascia ML, Rucci E, Piemontese MR, D'Agruma L, Persico AM.** 2007. HOXA1 gene variants influence head growth rates in humans. Am J Med Genet B Neuropsychiatr Genet **144:**388–390. http://dx.doi.org/10.1002/ajmg.b.30469.
91. **Tischfield MA, Bosley TM, Salih MA, Alorainy IA, Sener EC, Nester MJ, Oystreck DT, Chan WM, Andrews C, Erickson RP, Engle EC.** 2005. Homozygous HOXA1 mutations disrupt human brainstem, inner ear, cardiovascular and cognitive development. Nat Genet **37:**1035–1037. http://dx.doi.org/10.1038/ng1636.
92. **Bosley TM, Alorainy IA, Salih MA, Aldhalaan HM, Abu-Amero KK, Oystreck DT, Tischfield MA, Engle EC, Erickson RP.** 2008. The clinical spectrum of homozygous HOXA1 mutations. Am J Med Genet A **146:**1235–1240. http://dx.doi.org/10.1002/ajmg.a.32262.
93. **Silvis MR, Kreger BT, Lien WH, Klezovitch O, Rudakova GM, Camargo FD, Lantz DM, Seykora JT, Vasioukhin V.** 2011. α-Catenin is a tumor suppressor that controls cell accumulation by regulating the lo-

calization and activity of the transcriptional coactivator Yap1. Sci Signal **4:**ra33. http://dx.doi.org/10.1126/scisignal.2001823.
94. **Beverdam A, Claxton C, Zhang X, James G, Harvey KF, Key B.** 2013. Yap controls stem/progenitor cell proliferation in the mouse postnatal epidermis. J Investig Dermatol **133:**1497–1505. http://dx.doi.org/10.1038/jid.2012.430.
95. **Godwin AR, Capecchi MR.** 1998. Hoxc13 mutant mice lack external hair. Genes Dev **12:**11–20. http://dx.doi.org/10.1101/gad.12.1.11.
96. **Godwin AR, Stadler HS, Nakamura K, Capecchi MR.** 1998. Detection of targeted GFP-Hox gene fusions during mouse embryogenesis. Proc Natl Acad Sci U S A **95:**13042–13047. http://dx.doi.org/10.1073/pnas.95.22.13042.
97. **Tkatchenko AV, Visconti RP, Shang L, Papenbrock T, Pruett ND, Ito T, Ogawa M, Awgulewitsch A.** 2001. Overexpression of Hoxc13 in differentiating keratinocytes results in downregulation of a novel hair keratin gene cluster and alopecia. Development **128:**1547–1558.
98. **Ali RH, Habib R, Ud-Din N, Khan MN, Ansar M, Ahmad W.** 2013. Novel mutations in the gene HOXC13 underlying pure hair and nail ectodermal dysplasia in consanguineous families. Br J Dermatol **169:**478–480. http://dx.doi.org/10.1111/bjd.12302.
99. **Farooq M, Kurban M, Fujimoto A, Fujikawa H, Abbas O, Nemer G, Saliba J, Sleiman R, Tofaili M, Kibbi AG, Ito M, Shimomura Y.** 2013. A homozygous frameshift mutation in the HOXC13 gene underlies pure hair and nail ectodermal dysplasia in a Syrian family. Hum Mutat **34:**578–581. http://dx.doi.org/10.1002/humu.22271.
100. **Lin Z, Chen Q, Shi L, Lee M, Giehl KA, Tang Z, Wang H, Zhang J, Yin J, Wu L, Xiao R, Liu X, Dai L, Zhu X, Li R, Betz RC, Zhang X, Yang Y.** 2012. Loss-of-function mutations in HOXC13 cause pure hair and nail ectodermal dysplasia. Am J Hum Genet **91:**906–911. http://dx.doi.org/10.1016/j.ajhg.2012.08.029.
101. **Kang W, Tong JH, Chan AW, Lee TL, Lung RW, Leung PP, So KK, Wu K, Fan D, Yu J, Sung JJ, To KF.** 2011. Yes-associated protein 1 exhibits oncogenic property in gastric cancer and its nuclear accumulation associates with poor prognosis. Clin Cancer Res **17:**2130–2139. http://dx.doi.org/10.1158/1078-0432.CCR-10-2467.
102. **Muramatsu T, Imoto I, Matsui T, Kozaki K, Haruki S, Sudol M, Shimada Y, Tsuda H, Kawano T, Inazawa J.** 2011. YAP is a candidate oncogene for esophageal squamous cell carcinoma. Carcinogenesis **32:**389–398. http://dx.doi.org/10.1093/carcin/bgq254.
103. **Orr BA, Bai H, Odia Y, Jain D, Anders RA, Eberhart CG.** 2011. Yes-associated protein 1 is widely expressed in human brain tumors and promotes glioblastoma growth. J Neuropathol Exp Neurol **70:**568–577. http://dx.doi.org/10.1097/NEN.0b013e31821ff8d8.
104. **Steinhardt AA, Gayyed MF, Klein AP, Dong J, Maitra A, Pan D, Montgomery EA, Anders RA.** 2008. Expression of Yes-associated protein in common solid tumors. Hum Pathol **39:**1582–1589. http://dx.doi.org/10.1016/j.humpath.2008.04.012.
105. **Zhou Z, Zhu JS, Xu ZP, Zhang Q.** 2011. Lentiviral vector-mediated siRNA knockdown of the YAP gene inhibits growth and induces apoptosis in the SGC7901 gastric cancer cell line. Mol Med Rep **4:**1075–1082. http://dx.doi.org/10.3892/mmr.2011.543.
106. **Chen Q, Zhang N, Gray RS, Li H, Ewald AJ, Zahnow CA, Pan D.** 2014. A temporal requirement for Hippo signaling in mammary gland differentiation, growth, and tumorigenesis. Genes Dev **28:**432–437. http://dx.doi.org/10.1101/gad.233676.113.
107. **Lehn S, Tobin NP, Sims AH, Stal O, Jirstrom K, Axelson H, Landberg G.** 2014. Decreased expression of Yes-associated protein is associated with outcome in the luminal A breast cancer subgroup and with an impaired tamoxifen response. BMC Cancer **14:**119. http://dx.doi.org/10.1186/1471-2407-14-119.
108. **Matallanas D, Romano D, Yee K, Meissl K, Kucerova L, Piazzolla D, Baccarini M, Vass JK, Kolch W, O'Neill E.** 2007. RASSF1A elicits apoptosis through an MST2 pathway directing proapoptotic transcription by the p73 tumor suppressor protein. Mol Cell **27:**962–975. http://dx.doi.org/10.1016/j.molcel.2007.08.008.
109. **Oka T, Mazack V, Sudol M.** 2008. Mst2 and Lats kinases regulate apoptotic function of Yes kinase-associated protein (YAP). J Biol Chem **283:**27534–27546. http://dx.doi.org/10.1074/jbc.M804380200.
110. **Yuan M, Tomlinson V, Lara R, Holliday D, Chelala C, Harada T, Gangeswaran R, Manson-Bishop C, Smith P, Danovi SA, Pardo O, Crook T, Mein CA, Lemoine NR, Jones LJ, Basu S.** 2008. Yes-associated protein (YAP) functions as a tumor suppressor in breast. Cell Death Differ **15:**1752–1759. http://dx.doi.org/10.1038/cdd.2008.108.
111. **Tufail R, Jorda M, Zhao W, Reis I, Nawaz Z.** 2012. Loss of Yes-associated protein (YAP) expression is associated with estrogen and progesterone receptors negativity in invasive breast carcinomas. Breast Cancer Res Treat **131:**743–750. http://dx.doi.org/10.1007/s10549-011-1435-0.
112. **Chen H, Sukumar S.** 2003. HOX genes: emerging stars in cancer. Cancer Biol Ther **2:**524–525. http://dx.doi.org/10.4161/cbt.2.5.525.
113. **Cillo C, Cantile M, Faiella A, Boncinelli E.** 2001. Homeobox genes in normal and malignant cells. J Cell Physiol **188:**161–169. http://dx.doi.org/10.1002/jcp.1115.
114. **Abate-Shen C.** 2002. Deregulated homeobox gene expression in cancer: cause or consequence? Nat Rev Cancer **2:**777–785. http://dx.doi.org/10.1038/nrc907.
115. **Raman V, Martensen SA, Reisman D, Evron E, Odenwald WF, Jaffee E, Marks J, Sukumar S.** 2000. Compromised HOXA5 function can limit p53 expression in human breast tumours. Nature **405:**974–978. http://dx.doi.org/10.1038/35016125.
116. **Bitu CC, Destro MF, Carrera M, da Silva SD, Graner E, Kowalski LP, Soares FA, Coletta RD.** 2012. HOXA1 is overexpressed in oral squamous cell carcinomas and its expression is correlated with poor prognosis. BMC Cancer **12:**146. http://dx.doi.org/10.1186/1471-2407-12-146.
117. **Zhang X, Zhu T, Chen Y, Mertani HC, Lee KO, Lobie PE.** 2003. Human growth hormone-regulated HOXA1 is a human mammary epithelial oncogene. J Biol Chem **278:**7580–7590. http://dx.doi.org/10.1074/jbc.M212050200.
118. **Xiao F, Bai Y, Chen Z, Li Y, Luo L, Huang J, Yang J, Liao H, Guo L.** 2014. Downregulation of HOXA1 gene affects small cell lung cancer cell survival and chemoresistance under the regulation of miR-100. Eur J Cancer **50:**1541–1554. http://dx.doi.org/10.1016/j.ejca.2014.01.024.
119. **Cantile M, Scognamiglio G, Anniciello A, Farina M, Gentilcore G, Santonastaso C, Fulciniti F, Cillo C, Franco R, Ascierto PA, Botti G.** 2012. Increased HOX C13 expression in metastatic melanoma progression. J Transl Med **10:**91. http://dx.doi.org/10.1186/1479-5876-10-91.
120. **Svingen T, Tonissen KF.** 2003. Altered HOX gene expression in human skin and breast cancer cells. Cancer Biol Ther **2:**518–523. http://dx.doi.org/10.4161/cbt.2.5.441.