Arid1a regulates cell cycle exit of transit-amplifying cells by inhibiting the Aurka-Cdk1 axis in mouse incisor

Jiahui Du, Junjun Jing, Shuo Chen, Yuan Yuan, Jifan Feng, Thach-Vu Ho, Prerna Sehgal, Jian Xu, Xinquan Jiang and Yang Chai
DOI: 10.1242/dev.198838

Editor: Liz Robertson

Review timeline
Original submission: 18 November 2020
Editorial decision: 29 December 2020
First revision received: 23 February 2021
Accepted: 18 March 2021

Original submission

First decision letter

MS ID#: DEVELOP/2020/198838

MS TITLE: Arid1a regulates cell cycle exit of transit-amplifying cells through inhibiting the Aurka-Cdk1 axis in mouse incisor

AUTHORS: Jiahui Du, Junjun Jing, Shuo Chen, Yuan Yuan, Jifan Feng, Thach-Vu Ho, Xinquan Jiang, and Yang Chai

I have now received all the referees' reports on the above manuscript, and have reached a decision. The referees' comments are appended below, or you can access them online: please go to BenchPress and click on the 'Manuscripts with Decisions' queue in the Author Area.

As you will see, the referees express considerable interest in your work, but have some significant criticisms and recommend a substantial revision of your manuscript before we can consider publication. If you are able to revise the manuscript along the lines suggested, which may involve further experiments, I will be happy receive a revised version of the manuscript. Your revised paper will be re-reviewed by one or more of the original referees, and acceptance of your manuscript will depend on your addressing satisfactorily the reviewers' major concerns. Please also note that Development will normally permit only one round of major revision.

We are aware that you may be experiencing disruption to the normal running of your lab that make experimental revisions challenging. If it would be helpful, we encourage you to contact us to discuss your revision in greater detail. Please send us a point-by-point response indicating where you are able to address concerns raised (either experimentally or by changes to the text) and where you will not be able to do so within the normal timeframe of a revision. We will then provide further guidance. Please also note that we are happy to extend revision timeframes as necessary.

Please attend to all of the reviewers' comments and ensure that you clearly highlight all changes made in the revised manuscript. Please avoid using 'Tracked changes' in Word files as these are lost in PDF conversion. I should be grateful if you would also provide a point-by-point response detailing how you have dealt with the points raised by the reviewers in the 'Response to Reviewers' box. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

Reviewer 1

Advance summary and potential significance to field

In this manuscript, the authors show that Arid1a, a component of the SWI/SNF complex, is crucial for allowing for the continuous growth of the rodent incisor, with the most likely mechanism being that it forces transit-amplifying cells to exit the cell cycle and differentiate. The authors propose, though do not prove, that Arid1a normally functions by repressing cell cycle genes in the Aurka/Ccnb1/Cdk1 axis. Loss of Arid1a leads to inappropriate expression or maintenance of this pathway and hence continued proliferation of TACs without differentiation. This leads to defective and slowed incisor growth. To date, the mechanism behind continuous growth has been investigated, though it has not been clear how stem cells in the incisor niche transition from TACs to differentiated cell types. Findings from this paper provide a possible mechanism and point to avenues that can be examined in disease situations of tooth development that could be positively impacted by adjusting proliferation or differentiation of tooth stem cells. It should be noted that most of the data of this paper, which relies heavily on antibody staining, is well-prepared and convincing.

Comments for the author

The primary problem with the manuscript concerns the authors' assertion about Arid1a function. They show that Arid1a loss (in Gli1-CreER;Arid1afl/fl mice) leads to upregulation of multiple cell cycle genes. Focusing on the Aurka/Ccbn1/Cdk1 pathway, the authors show that the expression of Aurka, Ccnb1 and Cdk1 is reduced after a two week tamoxifen treatment using RNAScope and qPCR. They also use ChIP and chromatin from the proximal mouse incisor mesenchyme of control adult mice to show that Arid1a can bind to the "proximal region" of each gene, "suggesting that Arid1a may directly regulate the transcription of these genes (Fig. 6R)." (page12). These are convincing and the suggested interpretation of the results appears reasonable and cautious, reflecting the findings of the data. To be clear, the temporal analysis of gene expression after two weeks does not directly address an immediate regulation of these genes by Arid1a. Again, the word "suggest" takes this into consideration. However, at four points in the discussion, the authors move this tentative finding into a more factual finding simply by changing the wording.

1. Last sentence in first paragraph of discussion: "In the present work, we found that Arid1a binds the promoters of Aurka, Ccnb1 and Cdk1 and represses their gene transcription directly during the mitotic exit of TACs."
2. Last sentence of second paragraph of discussion: "In the present study, using the mouse incisor as a model, we found that Arid1a regulates cell cycle exit of TACs through the Aurka-Ccnb1-Cdk1 axis,…"
3. Fourth to last sentence in third paragraph of discussion: "Interestingly, the present study has discovered that Arid1a is highly expressed in TACs in the adult mouse incisor and works as a negative cell cycle regulator to repress the gene transcription of Aurka, Ccnb1 and Cdk1 directly."
4. Fourth to last sentence in last paragraph of discussion: "Furthermore, we have discovered that Arid1a binds the promoters of Aurka, Ccnb1 and Cdk1 and represses their gene transcription directly during the mitotic exit of TACs."

Again, the authors have only shown that gene expression goes up after two weeks of tamoxifen treatment (Arid1a deletion) and that in control incisor mesenchyme, Arid1a can bind to the proximal promoter. At no point do they show that changes in TAC exit are due to the binding of Arid1a to the promoters of Aurka/Ccbn1/Cdk1. The only way for the authors to prove this is to clone the region amplified by their qPCR primers into a Luciferase vector and then show that Arid1a can stimulate luciferase activity. It is somewhat surprising that this is not in the manuscript already. While this does not prove that these regions are responsible for Arid1a-mediated repression (which would require at least transgenesis, something outside the scope of this manuscript), it would at least prove that in vitro, the authors' model is reasonable. Also, the authors need to add the region that they are amplifying with the primers in the manuscript; the region should at least be listed relative to the start site of transcription and ideally also include the genomic coordinates. This is part of rigor and reproducibility of any ChIP study.

Smaller concerns:
1. The authors need to state with a reference that Sox2-Cre is only active in the ectoderm after birth (or at least in the oral cavity).
2. Ki67 is listed as both a TAC marker and a marker labeling cycling cells. The latter designation is more accurate while also explaining why it marks TACs so should be used in both locations in the text.
3. The color of "Ki67" in the header of the figure is off from Ki67 color in the figure and should be adjusted.
4. The histologic changes observed in Figure 2L-0 are not addressed in the text or legend. There is no explanation for the increased space in the odontoblasts, or their wavy nature. Also, what are the two humps in Figure 2O? It seems like there is a lot of data in this figure that is simply unclear or not covered but that could be informative to the phenotype of the mice.

Reviewer 2

Advance summary and potential significance to field

Arid1a is a core component of the SWI/SNF chromatin remodeling complex that plays critical roles in global transcriptional regulation during cell differentiation. This study uses the continuously growing mouse incisor as a model to investigate the roles of Arid1a in mesenchymal stem cell differentiation and tissue homeostasis. The authors found that Arid1a is expressed in all differentiated lineages but not in the Gli1+ epithelial and mesenchymal stem cells in the incisor tissues. Using a Gli1-CreER driver, they studied the effect of induced inactivation of Arid1a in incisor renewal in adult mice and found that the Arid1a-cko mice had defects in incisor growth. Histological and molecular marker (Dspp) analysis showed defect in odontoblast formation, resulting in differentiation of odontoblasts closer to the proximal base and lack of new preodontoblast cells derived from EdU-labeled transit amplifying cells. Lineage tracing result suggested a general inhibition of differentiation of the transit amplifying cells to both the odontoblast and pulp lineages, with vast increase in actively proliferating cells at the proximal end of the incisor tissues. The authors performed RNA-seq analysis of the proximal incisor tissues and found significant increase in expression of many cell cycle regulators, including Aurka, Ccnb1, and Cdk1. In situ hybridization showed, consistent with the vast increase in Ki67+ mesenchymal cells, a dramatic increase in the number of proximal mesenchymal cells expressing Aurka, Ccnb1, and Cdk1. Furthermore, pHH3 immunostaining showed that many of the EdU+ cells were undergoing active mitosis in the cko mutant incisor mesenchyme. ChIP-qPCR analysis showed that Arid1a is enriched at the promoter regions of the Aurka, Ssnb1, and Cdk1 genes in wildtype mice. In addition, the manuscript reported that treatment of the cko mice with the PP2A activator SMAP appeared to partly rescue odontoblast differentiation. The authors concluded that Arid1a regulates incisor tissue homeostasis through promoting cell cycle exit and differentiation of the transit amplifying cells by directly repressing the gene transcription of Aurka, Ccnb1, and Cdk1.

Comments for the author

(1) The data clearly demonstrate a failure of the Arid1a cko TACs to differentiate into odontoblasts and pulp mesenchyme cells. While the phenotype manifested as increased number of cycling and dividing TACs, the data are insufficient to conclude that Arid1a regulates TAC cell cycle exit through direct repression of the Aurka-Cdk1 axis. It could be possible that the TACs are stuck in the undifferentiated state due to failure of activation of differentiation programs or blocked response to differentiation signals. The effect of Arid1a inactivation of the expression and function of major odontoblast lineage specifiers need to be investigated.
(2) While the data presented in the Results section showed increased number of cycling and mitotically active mesenchymal cells in the Arid1a cko incisor and possible binding of Arid1a at the promoters of Aurka, Ccb1, and Cdk1 in control incisor tissues (the data showed Arid1a binding to every promoter tested – it would be good to show lack of Arid1a in some other gene promoters), of which the authors suggested "that Arid1a may directly regulate the transcription of these genes" (Page 12). However, in the Abstract (Page 2), Introduction (Page 5), and Discussion (multiple similar statements on Pages 13, 15, 16) sections, the manuscript presents the data as a conclusion that Arid1a promotes cell cycle exit and differentiation of TACs "by directly repressing gene transcription of Aurka, Ccnb1, and Cdk1". The data presented are only correlative/suggestive,

which could raise the hypothesis but no data have been presented to prove “direct transcriptional repression”. Additional experiments will be needed to test whether Arid1a indeed could directly represses transcription of those genes. Furthermore, Arid1a has been shown to participate in activation of gene expression in other contexts. It is not clear how Arid1a could directly repress gene transcription and why the effect is specific to the dental mesenchyme but not in the dental epithelium, which should be discussed.
(3) The data indicate that the increase in mitotic cells accounts for most of the increased Ki67+ cells and there was no increase in the number of TACs undergoing DNA synthesis. Does not suggest arrest of cell cycle in mitosis? Did the authors detect an overall reduction in the number of progeny cells, irrespective of cell cycle status, from the MSCs within 1 – 2 weeks of tamoxifen treatment? If the TACs are arrested in mitosis, then the manuscript should make it clear that the inhibition of odontoblast differentiation in the cko mice is secondary to the mitotic arrest of TACs.
(4) The SMAP treatment result is very interesting but not analyzed in detail to know what SMAP has done to TACs and preodontoblast differentiation. Did SMAP treatment significantly reduce the number of PHH3+ cells despite the overexpression of the Aurka-Cdk1 axis genes? SMAP could have many targets, but analysis of direct effects on the dental mesenchyme cells would enhance the story.
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First revision

Author response to reviewers' comments

Both reviewers commented very positively on our work. We have revised the manuscript where appropriate to address the criticisms raised in the reviews. The authors are greatly appreciative of all suggestions offered by the reviewers and feel that our revised manuscript is greatly improved through this review process. The following are our point-by-point responses to reviewers’ comments:

Reviewer 1: Advance summary and potential significance to field
In this manuscript, the authors show that Arid1a, a component of the SWI/SNF complex, is crucial for allowing for the continuous growth of the rodent incisor, with the most likely mechanism being that it forces transit-amplifying cells to exit the cell cycle and differentiate. The authors propose, though do not prove, that Arid1a normally functions by repressing cell cycle genes in the Aurka/Ccnb1/Cdk1 axis. Loss of Arid1a leads to inappropriate expression or maintenance of this pathway and hence continued proliferation of TACs without differentiation. This leads to defective and slowed incisor growth. To date, the mechanism behind continuous growth has been investigated, though it has not been clear how stem cells in the incisor niche transition from TACs to differentiated cell types. Findings from this paper provide a possible mechanism and point to avenues that can be examined in disease situations of tooth development that could be positively impacted by adjusting proliferation or differentiation of tooth stem cells. It should be noted that most of the data of this paper, which relies heavily on antibody staining, is well-prepared and convincing.

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qPCR. They also use ChIP and chromatin from the proximal mouse incisor mesenchyme of control adult mice to show that Arid1a can bind to the "proximal region" of each gene, “suggesting that Arid1a may directly regulate the transcription of these genes (Fig. 6R).” (page12). These are convincing and the suggested interpretation of the results appears reasonable and cautious, reflecting the findings of the data. To be clear, the temporal analysis of gene expression after two weeks does not directly address an immediate regulation of these genes by Arid1a. Again, the word “suggest” takes this into consideration. However, at four points in the discussion, the authors move this tentative finding into a more factual finding simply by changing the wording.

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We thank the reviewer for this comment. To confirm our findings, we performed dual luciferase assays to validate the gene regulation function of Arid1a at the promoters of Aurka, Ccnb1 and Cdk1. We found that the knockdown of Arid1a in the ST2 mesenchymal stromal cell line using siRNA leads to significant upregulation of promoter activities of Aurka, Ccnb1 and Cdk1 (Fig. 7A), suggesting that Arid1a participates in their transcriptional repression, most likely at the promoter regions. We added the genomic locations of these regions amplified with the ChIP-qPCR primers to our revised manuscript (Fig. 7B and Fig. S9).

Detailed and comprehensive analysis of chromatin accessibility changes after loss of Arid1a in the mouse incisor and investigation of other potential transcription modulation functions of Arid1a at other regulatory elements, besides promoter regions, remain to be done in the future. Several recent studies reported the emerging role of ARID1A in regulating enhancer-mediated gene expression in human colorectal cancer (Mathur et al., 2017) and neuroblastoma cells (Shi et al., 2020) and in preventing super-enhancer hyperactivation in endometrial epithelia (Wilson et al., 2020). These findings highlight the importance of Arid1a in homeostasis of multiple tissues as well as its context-dependent character. Therefore, besides the additional data in the Results section, we have modified our discussion to suggest that Arid1a may regulate Aurka/Ccnb1/Cdk1 to control the fate of TACs in the adult mouse incisor and incorporated the considerations noted above in the Discussion section.

Smaller concerns:
1. The authors need to state with a reference that Sox2-Cre is only active in the ectoderm after birth (or at least in the oral cavity).

We appreciate this suggestion. We have stated this point with associated references in our revised manuscript as follows: "Previous studies have shown that Sox2+ cells are epithelial stem cells in the adult mouse incisor that can contribute to all epithelial cell lineages (Arnold et al., 2011, Juuri et al., 2012, Juuri et al., 2013)."

2. Ki67 is listed as both a TAC marker and a marker labeling cycling cells. The latter designation is more accurate while also explaining why it marks TACs so should be used in both locations in the text.

We thank the reviewer for this suggestion. We have made the text consistent and now refer to Ki67 as a marker of cycling cells in our revised manuscript.

3. The color of "Ki67" in the header of the figure is off from Ki67 color in the figure and should be adjusted.

We appreciate this suggestion. We have adjusted the color of Ki67 in the header of the figure in our revised manuscript.

4. The histologic changes observed in Figure 2L-0 are not addressed in the text or legend. There is no explanation for the increased space in the odontoblasts, or their wavy nature. Also, what are the two humps in Figure 2O? It seems like there is a lot of data in this figure that is simply unclear or not covered but that could be informative to the phenotype of the mice.

We thank the reviewer for this comment. In our revised manuscript, we added associated description of the histologic changes of Gli1-CreER;Arid1afl/fl mice (Figure 2L-O) in the Results section and figure legend. To better show that loss of Arid1a results in severely disorganized dentin in the dental pulp cavity, we have included low magnification H&E staining of incisors from control and Gli1-CreER;Arid1afl/fl mice 3 months after induction (Fig. S2).

Reviewer 2 Advance summary and potential significance to field
Arid1a is a core component of the SWI/SNF chromatin remodeling complex that plays critical roles in global transcriptional regulation during cell differentiation. This study uses the continuously growing mouse incisor as a model to investigate the roles of Arid1a in mesenchymal stem cell differentiation and tissue homeostasis. The authors found that Arid1a is expressed in all differentiated lineages but not in the Gli1+ epithelial and mesenchymal stem cells in the incisor tissues. Using a Gli1-CreER driver, they studied the effect of induced inactivation of Arid1a in incisor renewal in adult mice and found that the Arid1a-cko mice had defects in incisor growth. Histological and molecular marker (Dspp) analysis showed defect in odontoblast formation, resulting in differentiation of odontoblasts closer to the proximal base and lack of new preodontoblast cells derived from EdU-labeled transit amplifying cells. Lineage tracing result suggested a general inhibition of differentiation of the transit amplifying cells to both the odontoblast and pulp lineages, with vast increase in actively proliferating cells at the proximal end of the incisor tissues. The authors performed RNA-seq analysis of the proximal incisor tissues and found significant increase in expression of many cell cycle regulators, including Aurka, Ccnb1, and Cdk1. In situ hybridization showed, consistent with the vast increase in Ki67+ mesenchymal cells, a dramatic increase in the number of proximal mesenchymal cells expressing Aurka, Ccnb1, and Cdk1. Furthermore, pHH3 immunostaining showed that many of the EdU+ cells were undergoing active mitosis in the cko mutant incisor mesenchyme. ChIP-qPCR analysis showed that Arid1a is enriched at the promoter regions of the Aurka, Ssnb1, and Cdk1 genes in wildtype mice. In addition, the manuscript reported that treatment of the cko mice with the PP2A activator SMAP appeared to partly rescue odontoblast differentiation. The authors concluded that Arid1a regulates incisor tissue homeostasis through promoting cell cycle exit and differentiation of the transit amplifying cells by directly repressing the gene transcription of Aurka, Ccnb1, and Cdk1.

Reviewer 2 Comments for the author
(1)The data clearly demonstrate a failure of the Arid1a cko TACs to differentiate into odontoblasts and pulp mesenchyme cells. While the phenotype manifested as increased number of cycling and dividing TACs, the data are insufficient to conclude that Arid1a regulates TAC cell cycle exit through direct repression of the Aurka-Cdk1 axis. It could be possible that the TACs are stuck in the undifferentiated state due to failure of activation of differentiation programs or blocked response

to differentiation signals. The effect of Arid1a inactivation of the expression and function of major odontoblast lineage specifiers need to be investigated.

We thank the reviewer for this comment. We agree with the possibility that the TACs may be stuck in the undifferentiated state due to failure of activation of differentiation programs. Previous studies have shown that BMP (Shi et al., 2019) and WNT signaling (An et al., 2018, Jing et al., 2021) are indispensable for the odontoblast differentiation of TACs in the adult mouse incisor. We did not find any apparent effect on them, as indicated for BMP signaling by the expression levels of p-Smad1/5/9 (Fig. S8A-D) or for WNT signaling by Ccnd1 and Axin2 (Fig. S8E-L). We also evaluated the expression level of Klf4, a critical transcription factor for odontoblast differentiation in the mouse molar (Feng et al., 2017), and did not find any apparent effect on its expression level in the pre-odontoblast and odontoblast region after loss of Arid1a (Fig. S8M-P). These results suggested that the defective TAC cell cycle exit after loss of Arid1a is most likely cell-autonomous and the odontoblast differentiation defect is secondary. We have incorporated these findings into our Results section.

(2)While the data presented in the Results section showed increased number of cycling and mitotically active mesenchymal cells in the Arid1a cko incisor and possible binding of Arid1a at the promoters of Aurka, Ccb1, and Cdk1 in control incisor tissues (the data showed Arid1a binding to every promoter tested – it would be good to show lack of Arid1a in some other gene promoters), of which the authors suggested "that Arid1a may directly regulate the transcription of these genes" (Page 12). However, in the Abstract (Page 2), Introduction (Page 5), and Discussion (multiple similar statements on Pages 13, 15, 16) sections, the manuscript presents the data as a conclusion that Arid1a promotes cell cycle exit and differentiation of TACs "by directly repressing gene transcription of Aurka, Ccnb1, and Cdk1". The data presented are only correlative/suggestive, which could raise the hypothesis but no data have been presented to prove "direct transcriptional repression". Additional experiments will be needed to test whether Arid1a indeed could directly represses transcription of those genes. Furthermore, Arid1a has been shown to participate in activation of gene expression in other contexts. It is not clear how Arid1a could directly repress gene transcription and why the effect is specific to the dental mesenchyme but not in the dental epithelium, which should be discussed.

We thank the reviewer for this comment and agree with it. Referring to the literature (Chandler et al., 2013), the promoter control element from a silent (Ins1) gene was used as negative genomic control in our revised manuscript (Fig. 7B). To confirm our findings, we performed dual luciferase assays to validate the gene regulation function of Arid1a at the promoters of Aurka, Ccnb1 and Cdk1. We found that the knockdown of Arid1a in the ST2 mesenchymal stromal cell line using siRNA leads to significant upregulation of the promoter activities of Aurka, Ccnb1 and Cdk1 (Fig. 7A), suggesting that Arid1a participates in their transcriptional repression most likely at promoter regions.
Previous studies have shown that Arid1a functionally binds gene promoters and negatively or positively affects the accessibility of transcription complexes such as RNA polymerase II and transcription repressors or activators (Wilson et al., 2019, Liu et al., 2020, Suryo Rahmanto et al., 2016). E2F4 is a transcriptional repressor of cell cycle genes at the promoters and Arid1a has been reported to interact with E2F4 in murine MC3T3-E1 cells and mouse liver (Nagl et al., 2007, Sun et al., 2016). Referring to the published ChIP-seq data on E2F4 (MacIsaac et al., 2010) and Arid1a (Gatchalian et al., 2018, Sun et al., 2016), we found that E2F4 and Arid1a share similar patterns of promoter occupancy at Aurka, Ccnb1 and Cdk1. We further confirmed the interaction of Arid1a and E2F4 in the proximal incisor mesenchyme using co-immunoprecipitation (Fig. S10), which suggested that E2F4 may participate in the transcription repression of Aurka, Ccnb1 and Cdk1 as a potential co-repressor with Arid1a. Detailed and comprehensive analysis of chromatin accessibility changes after loss of Arid1a in the mouse incisor and the other potential co-factors that facilitate the cell cycle regulation of Arid1a at other regulatory elements, besides promoter regions, remains to be done in the future. As noted in our response to Reviewer 1, several recent studies reported the emerging role of ARID1A in regulating enhancer-mediated gene expression in human colorectal cancer (Mathur et al., 2017) and neuroblastoma cells (Shi et al., 2020) and in preventing super-enhancer hyperactivation in endometrial epithelia (Wilson et al., 2020). These findings highlight the importance of Arid1a in homeostasis of multiple tissues as well as its context-dependent character. Interestingly, loss of Arid1a leads to significant upregulation of Aurka, Ccnb1 and Cdk1 in the proximal region of the incisor mesenchyme, but not in the dental

epithelium, which may be explained by the dominant regulatory role of Arid1a in the mesenchyme during incisor homeostasis; there might be mechanisms in the epithelium that can compensate for the loss of Arid1a. We have incorporated these findings and considerations in the Results and Discussion sections.

(3)The data indicate that the increase in mitotic cells accounts for most of the increased Ki67+ cells and there was no increase in the number of TACs undergoing DNA synthesis. Does not suggest arrest of cell cycle in mitosis? Did the authors detect an overall reduction in the number of progeny cells, irrespective of cell cycle status, from the MSCs within 1 – 2 weeks of tamoxifen treatment? If the TACs are arrested in mitosis, then the manuscript should make it clear that the inhibition of odontoblast differentiation in the cko mice is secondary to the mitotic arrest of TACs.

We appreciate this suggestion. Indeed, we found that loss of Arid1a leads to TACs arrested in mitosis and an overall reduction in the number of progeny cells from the MSCs 2 weeks after tamoxifen treatment (Fig. 3F-H). We agree that the inhibition of odontoblast differentiation in the cko mice is likely secondary and have made this point clear in our revised Results section.

(4) The SMAP treatment result is very interesting but not analyzed in detail to know what SMAP has done to TACs and preodontoblast differentiation. Did SMAP treatment significantly reduce the number of PHH3+ cells despite the overexpression of the Aurka-Cdk1 axis genes? SMAP could have many targets, but analysis of direct effects on the dental mesenchyme cells would enhance the story.

We thank the reviewer for this suggestion. Protein phosphatase 2A (PP2A) is one of the most critical phosphatases involved in cell division. It can dephosphorylate CDK1 substrates and promote mitotic exit (Wlodarchak and Xing, 2016). When we used the PP2A activator SMAP to counteract the overactivation of the Aurka-Ccnb1-Cdk1 axis, the number of PHH3+ cells after loss of Arid1a was reduced significantly (Fig. 6F-L). We have incorporated these findings into the Results section.

(5)Each section of the manuscript, including the Abstract, Results, and Discussion sections stated that the over-activated mitotic TACs provide feedback that leads to reduction in the MSC population. The Discussion section suggests further a negative feedback mechanism. However, the reduction of MSCs could be due to systemic effects of the reduced odontoblast differentiation and incisor growth, which ultimately signals to the MSCs to generate more TACs than usual and deplete the MSCs in the process, rather than a direct feedback from TACs to the MSCs. In addition, the cko mutants also showed defects in the proximal incisor structures that likely could disrupt the MSC niche. For example, Fig. 2M suggests disruption of the integrity of the cervical loop epithelium.

We thank the reviewer for this comment and we agree. We have corrected our text accordingly and incorporated this possibility into our revised Discussion section.

We greatly appreciate the editor and reviewers for these helpful comments, which have helped to improve our study. Thank you very much for your consideration.

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Second decision letter

MS ID#: DEVELOP/2020/198838

MS TITLE: Arid1a regulates cell cycle exit of transit-amplifying cells through inhibiting the Aurka-Cdk1 axis in mouse incisor

AUTHORS: Jiahui Du, Junjun Jing, Shuo Chen, Yuan Yuan, Jifan Feng, Thach-Vu Ho, Prerna Sehgal, Jian Xu, Xinquan Jiang, and Yang Chai
ARTICLE TYPE: Research Article

I am happy to tell you that your manuscript has been accepted for publication in Development, pending our standard ethics checks. Please note that the reviewer has identified a sentence that needs editing at the proof stage as well as expressing a concern about the size of the font being too small in one of the figures. You might want to edit the Figure as well to improve the labelling?

Reviewer 1

Advance summary and potential significance to field

The authors have addressed almost every concern raised by the first set of reviews. The addition of luciferase assays addresses concerns about whether Arid1a downregulates Aurka, Ccnb1, Cdk1, with further analysis of E2F uncovering additional possibilities for gene regulation. In addition, the description of the histology is vastly improved. There is so little to criticize that all I can say is that the following portion of a sentence on Pg. 12 needs to be edited:

"suggesting the cell cycle exit defect may primarily affected cells undergoing mitosis"

I am concerned that the font of the time lines may be very small after the figures undergo processing by Development (unless every figure is its own page), though that is post-submission production issue.

Comments for the author

See above

Reviewer 2

Advance summary and potential significance to field

same comment as on the original manuscript.

Comments for the author

The authors have done an excellent job responding to the reviewers' comments and added new experimental data that addressed critical concerns. The revised manuscript is greatly improved over the original version. I have no more concerns.