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RE: DEVELOPMENTAL-CELL-D-18-00032
"A Fibroblast Growth Factor 20-expressing, Wnt-responsive progenitor populates the olfactory epithelium and regulates turbinate growth"

Dear David,

I'm again so sorry about the delay here, but I at last have comments from the last reviewer, and I am now enclosing the comments from all three reviewers made on your paper. As you will see, they refer to the potential interest of the study, but they also indicate that further work would be necessary for publication in Developmental Cell. A key point brought up by several of the reviewers is the unclear relationship between Fgf20 and Wnt in this context. There are also some questions about tissue specificity of the phenotypes -- or really, the specificity of the Cre lines. From my reading of the comments, it seems premature to proceed with the paper on the basis of the current results. That said, I would not rule out the possibility that data could be obtained that would address the reviewers' concerns in a way that would make reconsideration appropriate. However, please note that you will not be able to submit a revision without contacting me first to discuss the revisions you are able to make and whether reconsideration seems productive; if so, I will reactivate the manuscript file to enable resubmission.

Yours sincerely,
Masha

Masha Gelfand, Ph.D.
Scientific Editor, Developmental Cell

Reviewer comments:

Reviewer #1: In this study, the authors use olfactory epithelium (OE) to address the mechanism of horizontal expansion. The OE is a pseudostratified epithelium which acts as neurosensory organ and in which specialized skeletal projections called turbinates are instrumental in giving the animal an acute sense of smell. The OE is a polarized structure with neuronal cells and odorant receptors lined apically, and basal cells forming the inner lining, giving the organ its functionality. Much is known about how progenitor populations from the basal layers expand and differentiate to give rise to neuronal structures. However, the mechanism by which the epithelial layer expands horizontally and the identity of progenitor populations that mediate such an expansion have remained controversial. This manuscript dissects the role of Sox2+/Fgf20+

cells at unique regions in the developing epithelia which can act as a supply to support expansion of the epithelia not only during embryonic development but also during postnatal life.

The work presented is of very high quality, as is typical for this lab. However, my main concern is that the connection with Wnt signaling, while interesting, is unclear. Wnts have been found to regulate Fgf20 in other contexts, but it is unclear if FGFs and WNTs are working in the same or in a parallel pathway. Another general issue in the manuscript is that the Fgf20GFP-Cre lineage is cumulative, and to make definitive conclusions one really would need an inducible system.

I have the following specific comments:

1. The data has been focused on negative folded regions or "neck" of the OE. These regions have a higher percentage of Sox2+Fgf10+ cells. The authors should determine using the ROS26AmTmG and ROSA26TdTomato reporters if these cells coincide with all the "neck" regions across the whole epithelia. Does the niche develop due to the specific shape of the cells at these "neck" regions?
2. The functional role of Fgf20 in progenitor cell fate maintenance has not been discussed sufficiently. In the Fgf20 mutants, the mesenchyme derived structures (cartilage and bone) are reduced in size and features as opposed to the controls. As mentioned in the current version of the manuscript, FGF20 primarily appears to govern the growth of mesenchymal cells, but this needs to be further elaborated in the Discussion section.
3. Lef/TCF-H2B; Fgf20GFP-Cre experiments and BFF-CKO experiments demonstrate the importance of Wnt/beta-catenin signaling. However, activation of Wnt/beta-catenin leads to a highly disorganized epithelial layer. Higher magnification images should be provided. If these cells lose tissue integrity and structure upon activation of Wnt/beta-catenin, they might exhibit hallmark properties of progenitors. The authors need to address this issue.

Minor comments:

4. At times on some of the images the text occludes the features of the images.
5. Some of the low magnification images are difficult to interpret without arrowheads or some other kinds of markers as a key.

Reviewer #2: The authors show FGF20 expression in the early lateral nasal cavity epithelium and later in the "necks" of the turbinates (protuberances bearing the sensory epithelium). Their lineage labellng shows FGF20 descendants populating nearly the entire mature olfactory epithelium. Conditional FGF20 knockouts had reduced turbinate size. Knockout of canonical Wnt activity (roughly coincident with FGF20 expression) reduced FGF20 and more severely reduced turbinate size and epithelial proliferation, also giving premature differentiation. Canonical Wnt gain-of-function showed the opposite phenotype (excess FGF20-expressing progenitor cells, excess growth of the epithelium, enlarged, misshapen turbinates).

This is a fairly straightforward paper and an interesting investigation of major developmental signals in growth and lineage regulation in the nasal turbinates, a relatively under-studied set of structures. The writing is a bit careless and needs clarification at many points (the minor points

listed below are for the first half of the paper - I gave up after that - detailed revision for the latter half is needed too). The conclusions are broadly supported except that it is stated that FGF20 signals to underlying mesenchyme whereas the data are also consistent with indirect effects of FGF perturbation affecting mesenchyme (e.g. by mechanical effects rather than signaling as such).

Additional experiments that would improve the paper would be more direct demonstration of an FGF response in the mesenchyme (e.g. expression of validated direct targets in normal and mutant tissue) and FGF signaling gain-of-function to complement the loss-of-function.

Minor points:

Title: "Wnt-responsive" should be "Wnt-expressing and -responsive"

General: The coinage FEP should be "FP" or just FGF20-positive. The E for epithelial or epithelium-spanning is redundant given that all cells in this epithelium are, of couse, epithelial and all are epithelium-spanning because it is pseudostratified not stratified.

p.4, para 2, last sentence "in real time" should be "over time"

p.4 and Fig.1C It is hard to see the non-stratification of the arrowhead-marked epithelium - the images themselves are a bit too highly pixellated. Could a higher-resolution image be provided?

p.4, penultimate para, "cells spanning the entire thickness of the OE"
There seems to be a confusion by the authors as to the meaning of the term "pseudo-stratified". In a pseudostratified epithelium ALL cells are epithelium-spanning. It is therefore confusing and misleading to distinguish the FGF20-positive cells as "epithelium-spanning". What they actually mean is that the FGF20-positive nuclei are at all apicobasal positions (just saying unstratified would be sufficient, although the additional clarification is fine).

p.5 "We term this cell population FEP (Fgf20-positive, epithelium-spanning progenitor) cells"
Calling these progenitor cells is premature in the narrative - it pre-empts the testing of the just-stated hypothesis. At least the authors should say that their coinage is based on results below.

p. 5, para.3 "we hypothesized that Fgf20 may be a marker for embryonic OE progenitors."
Repeats the "hypothesis" statement in the previous paragraph. Pick one or the other.

p.5, para 3, middle "The distribution of Fgf20+ cells and OMP+ ORNs in the c1 OE was linearly plotted" should be "The linear distribution of Fgf20+ cells and OMP+ ORNs in the c1 OE was plotted"

p.5 para.3 "Pde2+ necklace ORNs". What is "necklace"? Should it be "neck"?

p.5 para 4 "OE zones are described in Ressler et al., 1993; Vassar et al., 1993)"
On the basis of what are these zones distinguished in the cited papers?

p.5, para.4 "Given that OE expansion occurs in a dorsomedial-toventrolateral direction, the Fgf20GFP-Cre lineage is consistent with the idea that FEP cells horizontally expand the OE." This is a non-sequitur, or at least extremely poorly explained. What has the progression (not really direction - the epithelium points in many different directions) of expansion got to do with the observed labeling or the hypothesis that FEP cells do the expansion? One could argue that the presence of FGF20-negative cells and regions is NOT consistent with the hypothesis (or at least not with the simplest version of it, which is all the reader has). Presumably the authors are saying something about the very initial lateral and ventral enrichment of FEP cells at E12.5. A better explanation is necessary.

p.6 "Fgf20-null mice have a deficit in turbinate development"
"Have a deficit" (could mean absence) should be "have reduced turbinate surface area".

p.6, para 3 "Measurement of the surface of each turbinate to estimate size"
This is extremely unclear here and in the Methods. Points used to measure size could be better indicated in the figure (e.g. panels B')

p.7, para 4 "H2B-GFP was detected…most brightly in negatively curved OE at E17.5 (Figure 4D)"
This statement is not supported by the data. The brightest GFP is sometimes at the curved regions but is as strong or stronger in the flat walls flanking these curved regions.

Etc.

Reviewer #3: In this study, Yang et al investigate the role of Fgf20 expressed by olfactory epithelium progenitors, which they call Fgf20-positive, epithelium-spanning progenitors or FEPS, in the horizontal expansion of the epithelium as well as the underlying cartilaginous turbinate structures. In principle this is an interesting story that has the potential to tie together signaling events responsible for epithelial development that also coordinate growth and morphogenesis of the epithelium's underlying structural scaffold. However, I have a several major concerns that lead me to question whether the experiments in their current form support the authors' main conclusions.

My main concerns center on the general question of whether the genetic tools and perturbations employed are as specific as the authors claim. For example, no validation is provided to demonstrate the fidelity of the Fgf20-GFPcre transgene in the olfactory epithelium (OE) itself. There are also a number of known markers of OE progenitor cells that might have been used to produce a more complete characterization of these cells. Moreover, the undetectable levels of either the native mRNA/protein as well as the transgene reporter raises a serious question as to whether Fgf20 expressed by the OE progenitor cells could actually be active enough to serve as a developmental signal in the sub-mucosal tissue. It's certainly possible, but given these concerns a more careful analysis is warranted.

The phenotypes of the various Fgf20 and beta-catenin knockouts also seem open to

interpretation. For example, the Fgf20 knockout results in only a modest 15% reduction in overall surface area, a phenotype obtained with a germline knockout. Thus the contribution of Fgf20 to turbinate development seems modest at best, and it's further difficult to rule out that the effect, however modest, may be due to loss of function outside the OE itself. Similarly, the authors argue that canonical wnt signaling lies upstream of Fgf20, yet both beta-catenin knockouts demonstrate more severe phenotypes than the Fgf20 mutant. These observations indicate to me that the genetic perturbations employed here are not restricted to the OE progenitor cells in the way that the authors are presenting.

From Figure 6, the authors contend that beta-catenin knockout leads to premature differentiation and progenitor cell depletion. However the data shown, while suggestive, in fact are inconsistent with this conclusion, as no significant difference is found in the number of EdU+ progenitors at E14.5 nor were other known markers of OE progenitors tested. Similarly, the expression of the constitutively active beta-catenin seems to cause loss of mature cells in OE and other effects in underlying mesenchyme, but the phenotypes are too incompletely characterized to allow any meaningful conclusions to be drawn.