Reviewer’s report

Title: Apln-CreERT:mT/mG reporter mice as a tool for sprouting angiogenesis study

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Reviewer: Claudio Punzo

Reviewer's report:

The manuscript by Tao et al., shows the use of the Apelin-CreERT line for marking sprouting angiogenesis in the retina during development. The authors compare the Apelin-CreERT line to another Cre line (Cdh5) and show that the Apelin-Cre line is more specific for the newly forming vessels. Finally the authors show that they can enrich by FASC sorting for the Apelin-Cre expressing cells and confirm their finding by PCR.

The manuscript type is a technical advance and does not require any novel scientific findings. However, the technical advance is quite limiting. The authors crossed two mouse lines together that have been crossed before by others. None of the lines have been generated by the authors and what the authors show is that newly forming blood vessels are labeled by fluorescence as predicted. This has been demonstrated by the lab that generated the Apelin-cre line during development and and in the adult during tumor formation and under hypoxic conditions (PMID: 23797856; PMID: 25597280). The only advance here is that the authors show that the system works also in the retina during development, which is not much of an advance since the Cre-line is expressed in all newly forming blood vessels. What would have been technically more interesting is to add at least one example of the adult retina where angiogenesis is induced (e.g. ischemia, choroidal neovascularization, diabetic retinopathy, etc.). While having one example of adult angiogenesis would make the manuscript technically more interesting what is really needed are the following controls:

The authors should add at least one more example where Tamoxifen is injected at a later time point showing that only the far periphery is labeled during development or only the vessels that are the closest to the outer nuclear layer (cross-section or 3D image analysis) as vasculature development in the retina is not only central to peripheral but also from the ganglion cell layer to the outer plexiform layer. Having a later injection would make the difference between the two Cre-line more clear and demonstrate the usability of the system in the retina in a more convincing manner. Finally, as a control the authors should show that in the adult retina, with and without tamoxifen injection, GFP is not expressed as long as angiogenesis is not induced. That is obviously why having an adult where it is induced would have been nice as a positive control. Since this is a technical manuscript that presents a tool showing the utility of the tool for different retinal applications is important. Otherwise it appears to be a mere repeat of already published work.

With regards to the manuscript in general the background and discussion are presented in a way that makes the reader think that developing a tool to track newly sprouting blood vessels as a general approach is what is shown here. In particular for cancer research and other diseases
mentioned in the manuscript. However, the tool already exists and was not generated by the authors, the authors just test if it works in the retina. This is somewhat disingenuous and must be presented much clearer (e.g. last paragraph of background: page 5 line 5 says: "Here we present a new powerful Apln-CreERT line, ...." The authors did not generate this line but it certainly reads like that). The authors should stick to a story that talks about testing a tool that has been generated by others for its use in the retina.

Minor comments:
- There is no reference given for the Cre driver lines (Apelin-Cre & Cdh5-Cre) in the material and methods under animal. One has search in the text the reference for the Apelin-Cre line (#9). Curiously the sentence that presents the Cre line (#9) is presented in a manner as if there is a need to generate such line, although it exists. Finally, the reference (#9) is not the publication that originally presented the Apelin Cre line (although it is the same lab). The correct reference is PMID: 23797856 (mentioned already above).

- RNA purification: It appears that FASC sorting in paragraph above was done with fixed cells. Was the RNA extraction from FACS sorted cells also done with fixed cells? If so please clarify that in protocol. If not, why was fixation done in one case and not the other?

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

No

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

No

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

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