Reviewer's report

Title: IncompleteDll4/Notch Signaling Inhibition Promotes Functional Angiogenesis Supporting the Growth of Skin Papillomas

Version: 2  Date: 11 March 2015

Reviewer: Carrie Shawber

Reviewer's report:

The authors present a study that addresses the clinically significant concerns as to the affects of partial Dll4/Notch inhibition in tumor angiogenesis. They demonstrate that Dll4 heterozygosity leads to increased tumor angiogenesis correlating with increased tumor development and progression. The writing is clear and the discussion relevant to their study. The authors state that this occurs through the increase in VEGFR2 signaling in when Dll4 function is reduced. However, this is not clearly demonstrated and experiments need to be done to address this (see below).

Major Compulsory Revisions:

1) The Dll4+/- tumor angiogenic phenotype was described as “pronounced branching and thin interconnections (Line 249)”. The increased vascular density is clear in Dll4+/- tumors, but the latter description is not demonstrated in the images presented in Figure 2A. Either replace image to be more representative or adjust the text so that it is data not shown. Similarly, it is stated that the Dll4 tumors vessels have “reduced luminal diameters”. However this data is not presented.

2) Although there is an increase in VEGFR2/VEGFR1 ratio in circulation in Dll4+/- tumor baring mice, it is unclear why levels of both circulating VEGFR1 and VEGFR2 levels are down. If Dll4/Notch signaling in endothelial cells induces VEGFR1 and suppresses VEGFR2 expression, then circulating VEGFR2 levels should be increased in Dll4+/- and not decreased. Moreover, it is unclear how this data relates to specifically to the tumor vasculature as the mouse is globally heterozygous and all vessels are missing a copy of Dll4. Finally, it is unclear whether reduced circulating VEGFR1 levels is due to a decrease in endothelial expression of full-length VEGFR1 or sVEGFR-1 (soluble-Flt1). This is particularly important as Jagged1/Notch signaling has been shown to downregulate sVEGFR-1 (Kangsamaksin, Cancer Discovery 2015). Staining of the tumor tissues for endothelial VEGFR2 or VEGFR1 expression would help to clarify these concerns.

3) The observation that PDGFR-beta levels go up in Dll4+/- tumors is counterintuitive. PDGFR-beta is expressed in the aSMA+ VSMCs that are reduced in Dll4+/- tumors. Is this tumor expression of PDGFR-beta that is affected? Staining of tumor tissues would address this concern. It would be more
appropriate to look at PDGF-B transcript levels as it is expressed in endothelial cells. A reduction of PDGF-B levels would be consistent with the VSMC phenotype observed.

5) The authors infer a lot from reduced tumor volume in the sorafenib studies. As the focus is on the effects of Dll4+/- on tumor vessels, tumor sections should have been characterized for the vascular phenotype.

Minor Compulsory Revisions:

1) Alpha smooth muscle actin recognizes vascular smooth muscle cells and not pericytes that are aSMA negative. Please correct in the text: line 131.

2) Line 274: Typo: “VGFR2/VGFR1”

3) Figure 1A, upper left panel x-axis labels are cut off. This may be due to PDF rendering, but should be verified.

4) Figure 1B is a bit dark and should be lightened.

5) Figure 1C is described thoroughly in the text of the results (Lines 229-232), but it is unclear from the image and the figure legend how this translates. The image should be labeled and figure legend expanded to help the reader.

6) Figure 3A: No statistics are provided for this averaged data from sera analysis. Thus it is unclear what changes in expression are significant. Changes VEGF-A levels were not significant, whereas the decrease in circulating VEGFR1 and VEGFR2 is significant?

7) Figure 3B: What do the asterisks represent? This is not addressed in the figure legend. Labels in the figure should match the text of the figure legend, “WT vs Dll4+/+”.

8) Figure 4B bottom panel: The bars marking the significance are too crowded and an asterisk is missing.

Discretionary Revisions

1) As there is a reduction in aSMA+ VSMCs that has been shown to be regulated by EC Jagged1 signaling to VSMC Notch3, what does EC Jagged1 expression look like in the Dll4+-/ tumor vessels.

Level of interest: An article of outstanding merit and interest in its field

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:
Yes, I currently am an inventor on patents held by Columbia University, “Human NOTCH1 decoys” (WO2013052607), “Composition of humanized NOTCH fusion proteins and methods of treatment” (US Patent 20110008342 A1; ), and “Notch-based Fusion Proteins and Uses Thereof” (US Patent 7662919 B2). These patents are licensed with Eisai pharmaceuticals for the development of Notch inhibitors that target Notch in tumor cells and tumor endothelial cells. I received funds personally and to my lab from this license. These patents were developed with Jan Kitajewski.