Inhibition of the Notch Pathway Promotes Flap Survival by Inducing Functional Angiogenesis

To the Editor:

With interest, we read the recently published manuscript entitled "Inhibition of the Notch Pathway Promotes Flap Survival by Inducing Functional Angiogenesis."[1]

The authors conclude that intravenous injection of the γ secretase inhibitor (GSI) DAPT (N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl Ester) promotes skin flap survival and functional neovascularization by inhibition of the Notch signaling pathway.

Despite the authors' statement that little is known on the role of Notch in ischemia-induced angiogenesis, in-depth studies on the topic exist. It is known that a genetic lack of Notch signaling by the deficiency of the ligand Dll1 leads to detrimental tissue necrosis with loss of limbs in the hindlimb ischemia model.[2] In genetic gain and loss of function models of the Notch ligand Dll4 in different established models of ischemia, perfusion, or clinical outcome did not improve.[3] Similarly, DLL4 inhibition causes formation of a more numerous but disorganized capillary network in ischemic muscles.[4] Several groups have found that blockade of Notch signaling genetically and pharmacologically leads to disorganized and unproductive endothelial growth, which results in tumor hypoxia and necrosis.[5] Overall, the homeostasis of tissues by Notch signaling is complexly organized but a teamed effort by Notch ligands and Notch signaling restricts branching and is required to generate functional, perfused vessels.[6]

That pharmacological Notch inhibition should result in a beneficial clinical outcome in the reported surgical flap model is conceptually surprising and technically not sufficiently elaborated.

First and foremost, the γ-secretase cleaves several proteins, including Notch, E-cadherin, CD44, and erythroblastic leukemia viral oncogene homolog 4, which are all important modulators of angiogenesis.[7] The use of the GSI DAPT is thus unspecific, and the observed effects cannot be attributed to Notch signaling only.

This is the reason why relevant data on the role of Notch signaling in ischemia- and tumor-induced angiogenesis have been generated in genetic models of Notch signaling using effective γ-secretase inhibition only in addition.

In this context, it is stated that treatment with the GSI DAPT was given by intravenous injection. The substance is not soluble in aqueous solutions but needs to be solved in dimethyl sulfoxide. A more detailed protocol would be worth publishing alongside this manuscript. Furthermore, no evidence is provided that the chosen treatment protocol indeed inhibits Notch activity in vivo, for instance, data on Notch target gene expression in the flap model are not shown.

Interestingly, measurements of DLL4 protein levels in blood are presented as increased. However, DLL4 is a membrane-bound ligand, so detection of circulating DLL4 would indicate shedded ligands. The relationship to tissue expression levels are unclear and thus the relevance of the presented data.

We would like to point out that the model of Notch-DLL4 crosstalk in sprouting angiogenesis presented in Figure 1 is not timely and has recently been revised. The DLL4 expression in tip cells is only weakly modulated by vascular endothelial growth factor receptor 2 (VEGFR2). Notch signaling has little impact on VEGFR2 transcription but it strongly modulates VEGFR3.[8]

In the presented flap model, an increase of capillaries is noted with DAPT treatment. Because perfusion is not regulated at the level of capillaries,[9] the significance for flap perfusion is at best unclear. The presented micrographs provide no quantification, instead, on close inspection, raise the question whether DAPT treatment might lead to pathological angiogenesis. The conclusions that (1) vascular density is increased and (2) that functional neoangiogenesis is induced are thus not valid.

It is stated that this model represents an ischemic model. However, there is surgical wounding of the skin as well as assumed impairment of blood flow during lifting. No evidence has been provided that indeed the lifting skin model induces significant ischemia, and VEGF expression can occur independent of ischemia. The effects in this model are most likely compounded and difficult to interpret.

We suggest that gene names and protein names should be named according to international nomenclature, for instance, Dll4 or Vegfa for RNA and DLL4 or VEGF for proteins.

In addition, primer sequences for primers stated in the methods, such as for Dll4 RNA, should be reported.

Furthermore, it is surprising that the scientific data are represented without error bars and numbers for individual experiments. Although significance values are given, the presentation of data does not conform to standard practice. In addition, there seems to be confusion about the actual data presented in Figures 6 and 7. Although the figure legends report mean values, the text refers to median values of VEGF and DLL4, respectively.

Clearly, conclusions about the clinical implications of the study in the context of ischemia should be made with caution, after all its "notch so easy."

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Inhibition of the Notch Pathway Promotes Flap Survival by Inducing Functional Angiogenesis: Reply

Dear Editor:

We would like to thank Dr. Limbourg for her comments and insight regarding our article entitled "Inhibition Of The Notch Pathway Promotes Flap Survival By Inducing Functional Angiogenesis."[1]

Previous studies have investigated the functional importance of the Notch during ischemia-induced angiogenesis using various models, such as ischemic hindlimb and myocardium.[2,3] In

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