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Cell Death by Notch3-Dependent Notch Ligand Expression. Endothelial cells (ECs) play a vital role in organ development and vascular homeostasis. However, loss of endothelial cells promotes tumorigenesis by providing pre-metastatic niches for metastatic tumor cells. Here, we report that endothelial cell death is regulated by Notch3-Dll4 signaling. In ECs, Dll4 is expressed in distinct regions corresponding to areas of angiogenic sprouting. Notch3 receptor protein is expressed at the tip cell domain of vascular sprouts in vivo. EC death induced by intracellular  $\text{Ca}^{2+}$  overload was specifically inhibited by blocking Notch signaling. On the contrary, death induced by oxygen-glucose

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deprivation (OGD) was enhanced in hypoxic cocultured ECs by Notch3-ligand-Dll4 interaction. In mice with hyperactivated Notch3 or overexpression of Dll4 in ECs, tumor growth and post-operative metastasis were suppressed. We show that A673 human bone osteosarcoma cells alter the expression of Dll4 in ECs via autocrine mechanism and promote tumor growth in mice. Collectively, our findings reveal a novel role for the Dll4-Notch3 signaling pathway as a key determinant of tumor neovascularization. Our study also highlights a potential therapeutic target for human tumor through modulation of Notch3-Dll4 signaling.

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