

# Endothelial Dll4-Notch signaling in tumor microenvironment: is there any hidden therapeutic opportunity?

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**Abstract:** Tumor microenvironmental cross-talk between endothelial and cancer cells plays an essential role in growth, progression and therapeutic outcome of cancer. In response to tumor cell-derived vascular endothelial growth factor (VEGF), the endothelial delta-like ligand 4 (Dll4) regulates angiogenesis and tumor growth via Notch signaling. However, in view of intriguing effects of emerging Dll4-Notch signaling in various tumor types, the missing links need to be elucidated for development of this signaling as a possible therapeutic target for cancer therapy.

**Keywords:** Tumor microenvironment; angiogenesis; vascular endothelial growth factor signaling; Dll4-Notch receptor cross-talk; cancer therapy



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Tumor growth beyond ~1-2 mm in diameter poses limitations to get an adequate supply of blood nutrients and oxygen. These essential requirements are fulfilled by the formation of new blood vessels in tumor primarily through hypoxia-induced vascular endothelial growth factor (VEGF) signalling (1). VEGF secreted by tumor cells, acts on capillary endothelial cells reaching to the tumor site and induces their survival, proliferation and migration to meet the growing demand of nutrients and oxygen for tumor (2-4). In response to VEGF-mediated signaling, endothelial cell enhances expression of delta-like ligand 4 (Dll4), which is one of the ligands of Notch 1 and 3 receptors (5). In the work of Ding *et al.*, Dll4 has been found to inhibit lung cancer cell proliferation (A549 and H460) through consequential activation of Notch1 receptor and PTEN up-regulation, a known tumor suppressor (6). Existence of such signaling derived from endothelial cells could reduce the proliferative quotient of tumor cells, so that the nutrient and oxygen availability in tumor mass can be diverted to endothelial cells for the formation of new blood vessels. Moreover, it becomes plausible to further identify the interplay of other signaling arms, which can sense the

achieved equilibrium between neovasculature and tumor cell population, to regulate Dll4-Notch signals in tumor microenvironment. 'Oxygen concentration' seems to be one of the modulators of such homeostasis during Notch1 signaling in lung tumor (7).

Available evidences suggest the regulatory effect of Dll4 on endothelial and tumor cell proliferation. Inhibition of Dll4 signaling has been found to promote tumor angiogenesis and vascular neoplasm in various tissue types suggesting an anti-angiogenic effect of Dll4 (8,9). Thus, it becomes imperative to understand the significance of such signal which is anti-angiogenic and anti-proliferative to tumor cell compartment. VEGF derived from tumor cells influences multiple aspects of angiogenesis including regulation of capillary sprouting (2). Therefore, autocrine action of Dll4 on endothelial cells in combination with the effect of VEGF from tumor cells might be possibly required for limiting capillary branching and supporting longitudinal growth of new blood vessels, which ultimately restore the proper supply of nutrients and oxygen in tumor interior. In consistence with this understanding, accumulating evidences showed that blockade of Dll4-Notch1 signaling

in tumors results in hypersprouting with non-functional vasculature (10). This notion has been supported by evidences elucidating that endothelial tip cells possess high Dll4 to limit the sprouting at the growing end whereas low Dll4 in stalk cells might support proliferation of the neighbouring tumor cells (11,12). It will be of clinically relevant to understand the mechanism underlying the maintenance of Dll4 gradient across the longitudinal axis of a growing blood vessel, which might include unidentified tumor microenvironmental factors including VEGF. In view of above discussion, tumor suppressive effect of Dll4-Notch signaling observed by Ding *et al.* could be partially attributed to the deregulated angiogenesis (6). Therefore, a detailed understanding about the autocrine and paracrine effects of Dll4 in tumor microenvironment is required to propose Dll4-Notch signaling as a possible therapeutic target for cancer therapy.

In addition, the work of Ding *et al.* suggested an insignificant role of soluble factors derived from endothelial cells in Dll4-mediated suppression of A549 proliferation (6). Dll4 has also been observed as a membrane-bound ligand, which requires direct cell-cell interaction for its action (13). This might explain why conditioned medium of endothelial cells did not produce the same effect as observed with co-culture system or with the addition of recombinant Dll4. Preceding explanation raises an interesting query about the consistency of endothelial-tumor cell interactions involving Dll4-Notch signaling in a growing tumor condition.

Dll4 has been shown to activate specifically Notch 1 and 3 receptors. Dll4-mediated Notch 1 activation was found to induce tumor suppressive effects in non-small cell lung carcinomas (NSCLCs) through PTEN up-regulation while its pro-tumorigenic function was observed in T-cell acute lymphoblastic leukemia (T-ALL) or mesothelioma by down regulating PTEN activity (6,14,15). It is apparent that the biological effect of Notch1 activation is dependent on the tumor type, which could be due to the differences in transcriptional cofactors of Notch1, a key molecular determinant of subsequent PTEN regulation. This offers an opportunity to identify the Notch 1-interacting molecules determining the expression of genes involved in tumor growth or suppression. Dll4 has also been shown to interact with Notch 3 receptor whose association was observed to facilitate tumor growth in colorectal cancer and T-ALL (16). The work of Ding *et al.* showed that Dll4 did not affect mRNA levels of Notch-2 and -3 in NSCLCs (6). However, it could be important to investigate the basal and Dll4-induced Notch 3 activity in different cancer types.

It is possible that the net effect of Dll4 on tumor growth would depend on the relative expression, activation and intracellular effects of Notch 1 versus Notch 3. Thus, the contrasting effects of Dll4-Notch signaling i.e. tumor growth suppressive or promotive, pose further challenges to be faced in clinical scenario.

It becomes relevant to pose a question that what types of lung cancer patients would be benefitted most from Dll4-Notch based therapy? Considering tumor suppressive effects of Notch1 via PTEN upregulation in NSCLCs, its therapeutic application would be adversely affected in the various subcategories of lung cancer where frequent occurrence of PTEN-inactivating mutations was observed (17). The specific activation of Notch1 by Dll4 mimetic could be beneficial in lung cancers which show diminished or very low level of PTEN expression but retain the functional copy of *PTEN*. Another opportunity of therapeutic implication arises from blocking the action of Dll4 in endothelial cells which lead to tumor growth inhibition through non-productive angiogenesis. Such approach would be beneficial in those lung cancer subtypes which exhibit innate or acquired resistance to anti-VEGF therapy (18).

It may be pertinent to mention that before considering the clinical validation of these possibilities, better characterization of Notch1 signaling in lung tumor and endothelial cells, and its possible cross-talk with other oncogenic pathways would be essential to ensure rationale use of targeting Dll4-Notch signaling for the improvement of lung cancer therapy. Research also needs to be focused on identifying potential Notch signaling-based molecular signatures that could predict therapeutic response of molecularly targeted agents specific for lung cancer.

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