Cancer Research Journal
2022; 10(2): 46-50
http://www.sciencepublishinggroup.com/j/crj
doi: 10.11648/j.crj.20221002.15
ISSN: 2330-8192 (Print); ISSN: 2330-8214 (Online)

The Role of DLL4-Notch-VEGFR2 Signaling Pathway in Tumor Angiogenesis

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To cite this article:

Jie Zhou, Xinxin Duan, Ting Xiong, Aixia Sui. The Role of DLL4-Notch-VEGFR2 Signaling Pathway in Tumor Angiogenesis. *Cancer Research Journal*. Vol. 10, No. 2, 2022, pp. 46-50. doi: 10.11648/j.crj.20221002.15

Received: June 3, 2022; **Accepted**: June 17, 2022; **Published**: June 27, 2022

Abstract: Tumor angiogenesis is the center of tumor growth and metastasis, and it increases the supply of nutrients and oxygen to tumors, thereby supporting tumor growth and progression. Vascular endothelial growth factor (VEGF) is a stimulator of angiogenesis and also plays a key role in the process of angiogenesis. Overexpression of VEGF is associated with tumor angiogenesis, promotion of tumor growth and reduced survival rate of patients. Notch signaling is a key pathway that regulates the response to angiogenesis stimulation during embryonic vascular development and postnatal angiogenesis, and is involved in multiple steps of angiogenesis. Dll4 is the only Notch ligand mainly expressed in endothelial cells, which can propagate by activating Notch signal and regulate tumor angiogenesis. Notch signaling pathway and VEGF pathway have both independent and synergistic effects, and Notch is necessary for VEGF-mediated vascular remodeling. The pathways cross each other and jointly regulate tumor angiogenesis. This review reviews the relationship between dLL4-notch-VEGFR2 signaling pathway composition, transduction and regulation and tumor angiogenesis. Combined blocking of DLL4-notch-VEGFR2 signaling pathway can significantly reduce vascular perfusion, resulting in vascular degeneration and reduced tumor survival. It can destroy the vascular system and survival ability of the primary tumor more than blocking alone, thus providing a new idea for the treatment of tumor, which is of great significance for the future anti-angiogenesis therapy.

Keywords: DLL4, Notch, VEGF, Angiogenesis

1. Introduction

Tumor cell growth requires oxygen and nutrients, and tumor angiogenesis is the formation of a new blood vessel network that penetrates into the growing tumor, providing oxygen and nutrients, and removing waste [1]. In the presence of hypoxia and oncogenes, the expression of antigenic factors is abnormal and the dynamic regulation of vascular formation is broken, leading to the proliferation of vascular endothelial cells and the formation of new blood vessels [2]. The growth of new blood vessels, called "angiogenesis", is a morphogenetic process that is the center of tumor growth and metastasis, which increases the supply of nutrients and oxygen to tumors, thereby supporting tumor growth and progression [3]. VEGF is an angiogenesis stimulating factor that can initiate angiogenesis, while DLL4/Notch signaling pathway plays a negative feedback role in tumor angiogenesis and prevents excessive angiogenesis. The coordination of the two pathways ensures that the formed blood vessels have a certain function and ensure oxygen and nutrients needed for tumor growth.

In angiogenesis, vascular buds arise from endothelial cells (apical cells) that travel from the core of the blood vessel (stem cells). Apical endothelial cells are different from stalk cells in morphology, molecule and function, and their emergence is crucial for the formation of vascular buds [4]. Adapical cells migrate along a taxis gradient toward antigenic stimuli, producing long, dynamic filamentous pseudopods with tyrosine kinase receptors, which they use to detect the environment for directional signals. These apical cells exhibit

no proliferative, highly mobile, and tubular phenotypes. Stalk cells follow apical cells and proliferate as the buds extend in response to environmental cues. Stem cells polarize and coordinate the formation of vascular lumens, and establish adherents and tight junctions to maintain the integrity of new shoots [3]. Apical cells, designated by a complex cross-dialogue between VEGF and NOTCH signaling pathways, have independent but synergistic functions in tumor angiogenesis, driving angiogenesis together.

2. DLL4 Ligand Structure

Dll4 is the only Notch ligand mainly expressed in endothelial cells, especially in arterial endothelial cells [5]. Different from other ligands, Dll4 is cell contact-dependent and can propagate through positive feedback mechanism through activation of Notch signal [6]. Dll4 gene is located on chromosome 15q14, which is a type I single transmembrane protein composed of 685 amino acids. Human Dll4 protein has 8 EGF-like repeat peptides in the extracellular segment, 4 potential glycosylation sites and 1 DSL site composed of 45 amino acids. It contributes to the binding of Dll4 with Notch1 and Notch4 [7]. In Dll4 heterozygous mice, the haploid mortality was insufficient due to defects in sprouting angiogenesis and arteriovenous formation. So far, Dll4 is the second gene causing hypo haploid death, and the first gene is vascular endothelial growth factor (VEGF), indicating that Dll4 and VEGF play an indispensable role in angiogenesis[6]. The blocking of DLL4 inhibits tumor growth by regulating tumor angiogenesis, which is characterized by promoting pathological activation of endothelial cells and excessive tumor vascular growth without function [8]. Deterioration of impaired vascular communication in tumor microvascular network leads to deterioration of functional shunt, which may be the main cause of tumor microcirculation dysfunction and local hypoxia, and ultimately inhibit tumor growth [9].

3. Notch Signaling Pathway

Notch signaling is highly conserved during evolution and contributes to the development of multiple tissues and organs, including the vascular system. In mammals, Notch signaling consists of four Notch receptors (Notch 1-4) and five ligands (Jagged1 and Jagged2 and Dll1, Dll3 and Dll4) [10]. Notch signaling pathway is a key regulator of tumor vascular development and generation [11], which is involved in multiple steps of angiogenesis, such as apical cell differentiation, endothelial cell proliferation and mature vascular structure formation [12]. The interaction of Notch signaling between adjacent endothelial cells is believed to coordinate the cooperative behavior of cells during angiogenesis [13]. Notch signaling pathway is initiated when the extracellular domain of Notch ligand expressed on the surface of signaling cells binds to the extracellular domain of Notch receptor expressed on adjacent cells (recipient cells) [14]. After binding to ligands, the receptor undergo conformational changes, which trigger proteolytic activation of the receptor, release the intracellular domain (NICD), translocation into the nucleus [6], and interact with transcription factor RBPJK/CSL and coactivator manipulation protein (MAML) to regulate the expression of target genes [4]. This leads to induction or down-regulation of a variety of downstream targets for Notch signaling, the best downstream targets include the basic helix-loop-helix family of transcriptional inhibitors, cleavage of hairy and enhancers (HECS-1, HECS-5 and HECS-7) and hes1-related (Hey-1, -hey 2 and Hey-L) protein families [3]. Notch signaling plays an important role in determining the differentiation of arterial and venous endothelial cells into mesodermal vascular cells, increasing the antigenic potential of endothelial cells. During endothelial cell development, Hey1 and Hey2 (Notch signaling target transcription factors) have been reported to stimulate arterial endothelial cell differentiation and inhibit venous endothelial cell differentiation [15]. Notch signaling pathway is a necessary signal to determine the fate of arterial cells during embryonic development, and it controls vascular development and pathological angiogenesis by regulating the selection of endothelial apical cells and stalk cells in neovascularization [10].

4. The Relationship Between VEGF and Tumor Angiogenesis

VEGF-A, VEGF-B, VEGF-C, VEGF-D and VEGF-E, as well as placental growth factor (PGF) and VEGFR-1, VEGFR-2 and VEGFR-3 are key regulatory factors in angiogenesis regulation. Generally VEGF-A, VEGFA is A key molecule to induce angiogenesis, which leads to proliferation, germination, migration and tube formation of endothelial cells. It can also increase vascular permeability, make plasma protein leak and form extravascular matrix, thus further improving the subsequent growth environment of endothelial cells [10]. There is not enough evidence to prove that VEGF-B plays a direct role in angiogenesis. However, a recent study showed that VEGFB binding with VEGFR1 induces adipose tissue angiogenesis through the VEGF/VEGFR2 pathway [16]. VEGF-C and VEGF-D play a role in the formation of new blood vessels and lymphatic vessels in tumor tissues. VEGF-E is also a potential angiogenesis factor. Vegfr-1 and VEGFR-2 are mainly distributed on the endothelial surface of tumor vessels and regulate tumor angiogenesis. Vegfr-3 is primarily found on the surface of lymphatic endothelium and is the primary receptor that induces lymph angiogenesis (the formation of new lymph angiogenesis from existing lymph angiogenesis), but it has also been demonstrated to respond to VEGF-C in tumor angiogenesis [3].

Overexpression of VEGF in many solid tumors is associated with tumor angiogenesis, promotion of tumor growth, and reduced patient survival. VEGF plays an important role in tumor angiogenesis by binding its receptors

VEGFR1 and VEGFR2, and VEGF blockade is believed to reduce tumor angiogenesis and thereby inhibit tumor growth [17]. Shuang Yu et al. demonstrated a low VEGFR1/VEGFR2 ratio in lung cancer tissues, that VEGFR1 expression is negatively correlated with tumor microvascular density (MVD), and that VEGFR2 is positively correlated with MVD, suggesting that VEGFR2 may promote tumor angiogenesis. These results suggest that VEGFR1 is a negative regulator of VEGF activity, while VEGFR2 is the main mediator of VEGF biological effects [18]. Vegfr-2 is significantly expressed in apical cells and promotes polarized expression of apical filamentous feet in response to the presence of VEGF, leading endothelial cells to be guided along an antigenic signaling gradient, thereby promoting angiogenesis [3]. During the initiation of angiogenesis, endothelial apex cell potential is determined by VEGFR1 and VEGFR2 levels, and endothelial cells with high VEGFR2 and low VEGFR1 levels have a better chance of occupying and maintaining the lead [19].

5. Interaction and Mechanism of DLL4-Notch-VEGFR2 Signaling Pathway in Angiogenesis

Dll4-notch signaling pathway plays an important role in angiogenesis, vascular remodeling, and arterial or venous regulation [6]. DLL4-mediated activation of Notch signaling in blood vessels leads to a process known as lateral inhibition, which is critical for regulating branch formation during angiogenesis [20]. In normal physiology, DLL4-Notch signaling regulates angiogenesis by regulating the number of apical endothelial cells. Inhibition of DLL4-Notch signaling pathway can increase the number of endothelial apical cells, stimulate the angiogenesis response, increase MVD, and lead to excessive germination and the generation of nonfunctional blood vessels, which are poorly perfused and dysfunctional and reduce effective blood flow, which increases tumor hypoxia and thus reduces tumor growth [5]. Two major changes after NOTCH occlusion may lead to functional defects in tumor vascular system: lumen formation disorder and vascular network disorder. Tumor vascular histology and imaging studies have revealed the transformation to smaller vessel diameter and low blood flow in anti-DLL4-treated tumors [21]. DLL4 blockade may worsen the impaired vascular communication in tumor microvascular network, leading to deterioration of functional shunt, which may be the main cause of tumor microcirculation dysfunction and local hypoxia [9]. Tumor growth retardation due to DLL4-Notch inhibition is associated with endothelial cell proliferation, migration, and subsequent paradoxical increase in tumor vascular density, but also with excessive branching, lacunar defects, and impaired parietal cell recruitment, all leading to nonfunctional vascular formation and subsequent tumor starvation [22]. However, overexpression of DLL4-Notch signaling pathway in endothelial cells can reduce tumor vascular density and increase blood perfusion, thereby promoting tumor growth. These results suggest that the regulation of DLL4/Notch signaling pathway is a bidirectional process of angiogenesis [23]. DLL4 overexpression reduces the number of blood vessels by reducing vascular density as a negative regulator of tumor angiogenesis, but drives tumor growth by improving vascular diameter and perfusion. Loss of DLL4 expression leads to excessive angiogenesis and sprouting, reduction of vascular luminal size, formation of chaotic vascular network, intensification of functional shunt and reduction of blood perfusion, thus inhibiting tumor growth [3].

Notch and VEGF pathways have independent but synergistic functions in tumor angiogenesis, driving angiogenesis together [24] and Notch is required for VEGF-mediated vascular remodeling [25]. D. GUO1 et al. demonstrated that Notch overexpression promotes tumor angiogenesis, at least in part through up-regulation of VEGF [24]. Endothelial cell germination and proliferation are induced by VEGF-A and its receptor VEGFR2, which mediates the expansion of vascular network and initiates tumor angiogenesis. In addition to VEGF regulating vascular development, vascular branching and dilation are controlled by cell activation of Notch signaling [20]. Notch signaling, especially the interaction of DLL4-Notch signaling, enables selection and differentiation between apical and stalk cells during the early embryonic stages of physiological and pathological angiogenesis. DLL4 is mainly expressed in apical endothelial cells [3]. Dll4-notch signaling pathway, as a negative regulator of vascular bud growth, inhibits apical cell formation in response to VEGF [26]. Notch regulates VEGFR expression. Mara E. Pitulescu et al. demonstrated that Notch inhibition induces upregulation of VEGF-A and VEGFR2, thereby promoting angiogenesis [13]. VEGF signaling can induce the expression of DLL4 in endothelial cells [27]. After stimulation with VEGF and subsequent induction of DLL4 expression in signal-receiving endothelial cells, induction of the DLL4-Notch signaling pathway in adjacent signal-receiving cells resulted in transcriptional inhibition of VEGFR-2 and its co-receptor NRP-1 in signal-receiving cells. Notch also upregulates the expression of soluble and full-length VEGF-induced receptor VEGFR-1. Therefore, the signal receiving cells were unable to form the apical cell phenotype that responds to VEGF and instead differentiated towards the stalk cell phenotype, while the signal sending cells formed the apical cell phenotype. The proliferation of endothelial cells is also restricted by DLL4-Notch signaling. When the DLL4-Notch signaling pathway is blocked, all endothelial cells can play a role in the stimulation of VEGF indiscriminately, most of them can form filamentous toe cells, and the germination, branching and fusion of endovascular tubes are significantly increased. For example, After VEGF stimulation, Notch signaling blocks VEGF-dependent proliferation in adjacent cells by reducing the phosphorylation of ERK1/2 and AKT, and cell growth is blocked in the G0/G1 phase of the cell cycle. Notch signaling also contributes to endothelial cell survival (e.g. by inducing the expression of the anti-apoptotic protein Bcl-2). Notch activates VEGFR3,

which responds to VEGF-C and protects against endothelial cell apoptosis [3].

In most tumor models tested, additional antitumor activity was observed in combination with anti-VEGF therapy. Since angiogenesis and germination after DLL4 blockade is still a VEGF-dependent process, DLL4 inhibition may increase the dependence of tumor microvascular system on VEGF-mediated survival signals, affect the remodeling of tumor blood vessels, and make them more mature and stable, thus increasing the vulnerability of tumor blood vessels to VEGF blockade. In tumors inhibited by DLL4-Notch, the hyper proliferative state of endothelial cells and the reduced protective effect of supporting cells on tumor endothelium may make tumor vessels more susceptible to drugs that selectively target proliferative cells. Blocking DLL4-Notch can enhance the efficacy of anti-VEGF in sensitive tumors [28]. Sonia L Hernandez et al. demonstrated that Notch blockade can disrupt endothelial/pericyte interactions, but only in the absence of VEGF can significant loss of vascular and perfusion occur, and subsequently induce tumor necrosis [29]. In the absence of VEGF, Notch occlusion can induce endothelial cell apoptosis in vivo and in vitro. This suggests that VEGF can rescue endothelial cells when Notch is blocked. Antibodies to DLL4 and Notch increase nonfunctional angiogenesis in subcutaneous tumor models. Inhibition of DLL4-notchs-VEGFR2 not only promotes the formation of nonfunctional blood vessels, but also reduces the promoting effect of VEGF on normal angiogenesis, resulting in a cumulative effect, which can significantly reduce vascular perfusion, lead to vascular degeneration, increase endothelial cell apoptosis, and block endothelial cell per cellular coverage without affecting tumor weight. However, it does reduce the viability of the tumor, and at the same time, blocking the vascular system and viability of the primary tumor can be more damaged than inhibition of these two pathways alone [30].

6. Conclusion

Tumor growth need oxygen and nutrients, anti-angiogenesis therapy depend on deprived of oxygen and nutrients to kill tumor cells, but in many preclinical tumor models, blocking VEGF pathway was not significant influence tumor growth and tumor angiogenesis, which suggests that many other factors or pathway in tumor angiogenesis and growth also plays an important role, Tumors that are resistant to VEGF blockers require treatment with other anti-tumor antigenic agents. Blocking Dll4/Notch in tumor angiogenesis inhibits tumor vascular remodeling or maturation. However, anti-VEGF therapy is a factor that shuts down tumorigenesis, so blocking DLL4-Notch is still effective in tumors that fail to respond to anti-VEGF therapy. Combined blockade of DLL4-notch-VEGFR2 can significantly reduce vascular perfusion, lead to vascular degeneration, and reduce tumor survival, which can destroy the vascular system and survival of the primary tumor more than blockade alone.

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