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Review Article

Treatment of bronchopulmonary dysplasia by vascular endothelial growth factor: the earlier the better?

Jinghua Luo[1,2], Yingjian Si[1], Jia Chen[1], Zhichun Feng[1]

[1]*Affiliated Bayi Children's Hospital, Clinical Medical College in PLA Army General Hospital, Southern Medical University, Beijing, China;* [2]*Department of Pediatric, The Second Affiliated Hospital of Nanchang University, Nanchang, Jiangxi, P.R. China*

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Abstract: Bronchopulmonary dysplasia (BPD) is a chronic lung disease that most commonly occurs in premature infants who have needed mechanical ventilation and oxygen therapy for acute respiratory distress, but can also occur in immature infants who have had few signs of initial lung disease. Vascular endothelial growth factor (VEGF) has been shown to play a central role in vascular development. VEGF is a potent endothelial cell-specific mitogen and survival factor that stimulates angiogenesis, promotes vessel remodeling, and enhances endothelial survival. VEGF signaling is absolutely critical for vascular development and embryonic survival, and appears to protect the lung against hyperoxia or cytokine-induced endothelial cell injury. Whether disruption of VEGF signaling impairs lung vascular growth and contributes to the pathogenesis of BPD has been uncertain. Since the establishment of "vascular hypothesis of BPD", vascular endothelial growth factor (VEGF) has been adopted as one of the means for the treatments of BPD. However, the time of using VEGF is not unified. In this review, we firstly introduced the definition of BPD, and then explored the pathology, roles and mechanisms of VEGF in BPD, and finally briefly summarized the timing of using VEGF to treat BPD.

Keywords: Bronchopulmonary dysplasia, vascular endothelial growth factor, endothelial cells, treatment

Introduction

Bronchopulmonary dysplasia (BPD) is the most common disease among surviving premature infants and is associated with poor outcomes of long-term lung maturity and neuro-development [1-4]. Fortunately, various treatments for BPD have been developed, greatly increasing the survival rate of premature infants. The "old" BPD focuses mainly on lung injury resulting from oxygen therapy and mechanical ventilation, while the "new" BPD, on abnormalities in the lung development [1, 5, 6]. Recent reviews have shown that pulmonary vascular disease has become the new frontier of BPD research [5, 7]. According to the "vascular hypothesis of BPD", disruption of angiogenesis during lung maturity could impair lung development by decreasing alveolarization and pulmonary arterial density [8]. VEGF is a major mediator of vascular permeability, endothelial cell proliferation and migration, which is very important in vasculogenesis and angiogenesis [9, 10]. Previous studies have demonstrated that the expression of VEGF mRNA and protein decreased in alveolar lavage fluid or peripheral blood in children with BPD or in animal models [11, 12]. Researchers have begun to explore the use of VEGF replacement therapy in BPD [13, 14]. However, the diagnosis of BPD is currently based on the need for supplemental oxygen for at least 28 days after birth, and BPD is classified into several grades according to the respiratory support required at 36 postmenstrual weeks [15, 16]. So will it be late to take VEGF at 36 postmenstrual weeks? Is it necessary to give VEGF treatment in 24 hours after birth? The earlier the better? With the above questions in mind, we reviewed the pathophysiological process of BPD, the mechanism of VEGF involved in angiogenesis and stabilization, and the status quo of VEGF application in the treatment of BPD, aiming to determine the right time of taking VEGF.

Definition of BPD

Bronchopulmonary dysplasia (BPD) was first defined by Northway and his coworkers in 1967 [17]. It was described as prolongation of the healing phase of respiratory-distress syndrome combined with a generalized pulmonary oxygen

toxicity involving mucosal, alveolar and vascular tissues. They stressed the need of neonates for the oxygen therapy 28 days after birth, presence of clinical symptoms and visible chest changes revealed by X-ray as the diagnostic criteria for BPD [18]. The incidence of BPD ranged from 6% to 57% between 1978 and 2015, depending on the definition chosen [19, 20]. "BPD" is an operational definition in which the treatment (oxygen therapy at 28th day or 36th week postmenstrual age) is used to define the disease [21], so with the improvement of treatment, the definition of BPD varies. According to Hine's review, the definition by Shennan and his coworkers was adopted in 45% of a total of 628 papers reviewed [22], the NICHD definition, in 30% [15] (**Table 1**), and the physiological definition indicated by the oxygen challenge test, in approximately 6%. BPD is associated with significant morbidity and mortality in the neonatal intensive care unit [23] and is also associated with worse long-term outcomes such as increased airway hyperresponsiveness in childhood, abnormal lung function in young adults, and potentially earlier come up of chronic obstructive pulmonary disease [21, 24, 25].

Fortunately, the introduction of antenatal steroids, natural surfactant therapy, lower supplemental oxygen concentrations and gentler ventilation techniques altered the clinical course and pathology exhibited by preterm infants [26, 27]. Vollsaeter and his coworkers compared preterm infants with a gestational age <28 weeks or a birth weight <1000 g in western Norway from 1999-2000 with those in 1991-1992. They found that for children with neonatal BPD, important lung function variables were better in EP1999-2000 than in EP1991-1992. In regression models, administration of antenatal corticosteroids and surfactant treatment improved the lung function in the EP1999-2000 [28] but failed to benefit others in a visible way [15]. This new kind of BPD focused more on the interruption of normal development than lung injury from oxygen therapy and mechanical ventilation. These consist of very low birth weight infants who initially have mild or no lung diseases but whose need for oxygen and ventilatory increase over the first several weeks of life [29]. Some authors have described that kind of BPD as a "new" BPD [15, 30].

Pathology of the new BPD

The lungs, together with the trachea, arise from the anterior foregut endoderm (the 4-7 week of gestation in humans). From 7 to 16 weeks' gestation, evagination of these epithelial cells result in the formation of the trachea and two lung buds and the beginning of the lung development at the embryonic stage. At this stage, the trachea separates from the esophagus [31-34]. In the course of the lung development, first the trachea is formed, which then generates the bronchial tree and finally the airways which is largely in parallel with the vasculature of the pulmonary circulation [35]. Subsequent lung development at different stages including the canalicular, saccular and alveolar structures generate the alveolar-gas exchange units [36]. The lung at 26 weeks of gestation is just at the canalicular stage and is of the saccular structure without alveoli, which does not not begin to develop in another 4 to 6 weeks [15]. About at 30 to 32 weeks, the lung is at the saccular stage. With the growth of terminal saccules, extensive vessels are generated, and then the secondary crests occur along with the loss and remodel of interstitial extracellular matrix [37]. Although alveoli appear in some infants at 32 weeks of gestation, they do not uniformly grow up to 36 weeks at the stage of alveolar, and they continue to grow at a slower rate during the first 2-3 years after birth [8]. Thus, premature births and the initiation of pulmonary gas exchange will interrupt the development of normal alveolar and distal vascular, thereby becoming the two major features of the new BPD [38]. The "old" BPD was characterized by severe lung injury, pronounced inflammation, lung edema, airway epithelial metaplasia, peribronchial fibrosis, and remarkable hypertrophy of airway and pulmonary vascular smooth muscle [17, 39]. However, the "new" BPD is characterized by alveolar hypoplasia (fewer and larger alveoli), thickened alveolar septa, dysmorphic pulmonary microvascular networks, mild hypertrophy of airway and vascular smooth muscle, accumulation of interstitial fluid, abnormal deposition of extracellular matrix components and an arrest of lung development at the late canalicular to early saccular stage [40].

We reviewed literatures over the past three decades and found the role of vascular dysplasia in new BPDs, which are receiving increasing attention. We summarized the pathological manifestations of the lungs in some animal models in the table (**Table 2**), to better show the pathological features of the new BPD. Soliman et al performed a prospective cohort

Table 1. Definition of BPD (NICHD consensus 2001)

Gestational Age	<32 wk	≥32 wk
Time point of assessment	36 wk PMA or discharge to home, whichever comes first	Time point of assessment: >28 days but <56 days postnatal age or discharge to home, whichever comes first
	Treatment with oxygen 21% for at least 28 d plus	
Mild BPD	Breathing room air at 36 weeks PMA or discharge, whichever comes first	Breathing room air at 56 days postnatal age or discharge, whichever comes first
Moderate BPD	Need for <30% oxygen at 36 weeks PMA or discharge, whichever comes first	Need for <30% oxygen at 56 days postnatal age or discharge, whichever comes first
Severe BPD	Need for ≥30% oxygen and/or positive pressure, (positive pressure ventilation or NCPAP) at 36 weeks PMA or discharge, whichever comes first	Need for ≥30% oxygen and/or positive pressure, (positive pressure ventilation or NCPAP) at 56 days PMA or discharge, whichever comes first

Definition of abbreviations: BPD bronchopulmonary dysplasia; NCPAP nasal continuous positive airway pressure; PMA postmenstrual age; PPV positive-pressure ventilation.

Table 2. Pathology of the BPD

Author	Model/human	Pathophysiological characteristics
Northway WH Jr et al. 1967 [17]	Human lung slices	Severe lung injury, pronounced inflammation, lung edema, airway epithelial metaplasia, peribronchial fibrosis, and marked airway and pulmonary vascular smooth muscle hypertrophy
Gorenflo M et al. 1991 [98]	Lung slices and barium angiogram	Decreased density of peripheral pulmonary arteries.
Cherukupalli K. et al. 1996 [99]	Human lung slices	a. Group I was a phase of acute lung injury; Alveolar hyaline membrane, alveolar epithelial necrosis. b. Group II the proliferative phase; Cell metaplasia, airway epithelium ulcer. c. Group III the phase of early repair; Extensive type II metaplasia, pulmonary fibroblasts rich in interstitial. d. Group IV the phase of late repair; Airway epithelium phosphorylation, bronchial smooth muscle fibrosis.
Husain A et al. 1998 [100]	Human lung slices	a. No surfactant therapy: alveolar septal fibrosis, partial to complete arrest in acinar development (alveolar saccular and alveolar). b. Use surfactant therapy: less phosphorus-like metaplasia.
Coalson JJ et al. 1999 [101]	Baboons appropriate oxygen (1-2 m)	Decreased pulmonary microvasular development and alveolarization
Bhatt AJ et al. 2001 [11]	Human lung slices	Alve-olar capillaries were often located in the interior of thickenedsepta. dilated and lacked extensive network or-ganization.
Coalson JJ 2003 [38]	Baboons and Clinical specimens	"Emphysematous" distal lung structure with fewer 51 lung units, areas of septal thickening, microvascular dysplasia/hypoplasia and inflammation.
De Paepe ME et al. 2006 [44]	Postmortem lung samples	The microvasculature of ventilated lungs appeared immature, retaining a saccular architectural pattern.
Velten M et al. 2010 [102]	C3H/HeN mice (85% O_2, 14 dprenatal LPS)	Decreased alveolar number and increased size.
O'Reilly M et al. 2014 [103]	Mouse (65% O_2, 7 d)	More smooth muscle; no effect on bronchiolar epithelium or collagen.
Firsova AB et al. 2014 [104]	Mouse (95% O_2, 5 d)	Airspaces were significantly enlarged.
Belcastro R et al. 2015 [105]	Rat lung (60% O_2, 14 d)	Impairments of lung cell proliferation, secondary crest formation, and alveologenesis.
Mankouski A et al. 2016 [2]	Sprague-Dawley rats (60% O_2, 14 d)	Decreased numbers of secondary crests and peripheral vessels.
Jiménez J et al. 2016 [106]	Rabbits	Fewer and larger alveoli with thicker walls, less developed distal airways and more inflammation.
Chou HC et al. 2016 [56]	Prenatal LPS (85% O_2, 14 d)	Reduced vascular density

study, from January 2007 to June 2010 at a single tertiary care center, with infants less than 32 weeks' gestation born to mothers with preeclampsia, and found that preeclampsia, an antiangiogenic state, is an independent risk factor of bronchopulmonary dysplasia (BPD) [41]. Baud et al found that angiogenesis blocked by vascular endothelial growth factor (VEGF)-Trap decreased the number of lung capillaries and enlarged the size of alveoli, which is similar to pathological manifestations of BPD [7, 42]. This suggests that angiogenesis plays an important role in alveolarization. It is noted that glucocorticoids are widely administered to accelerate the maturation of AEC2 cells and production of surfactant in premature babies, which appear to inhibit secondary septation and vascular development [33]. So far, the only consistent vascular findings in new BPD pathology are that the structural configuration of the distal microvasculature is abnormal, namely dysmorphic [37]. This kind of dysmorphica shows an abnormal distribution of alveolar capillaries in lungs, the vessels being far away from the air surface [43] and the dysmorphia being of a saccular architectural pattern [44].

The role of VEGF in BPD

VEGF family

Vascular endothelial growth factor (VEGF) is a multifunctional cytokine which plays a key role in many physiological (angiogenesis, growth and organ repair) and pathological (vascular disease) processes [45]. The VEGF gene is located on chromosome 6q21.3, and consists of eight exons and seven introns [46]. Multiple isoforms of VEGF, ranging from 121 to 206 amino acids [47], can be generated by alternative exon splicing, and these isoforms differ in their ability to bind heparin, which determines their bioavailability and may play distinct roles in angiogenesis during development [48-50]. In humans, VEGF is made up of five secreted glycoproteins which include VEGF-A, VEGF-B, VEGF-C, VEGF-D and placental growth factor (PlGF) [48]. VEGF-E is encoded by certain viruses and its gene is not contained within the human genome [51]. VEGF-A activates intracellular signaling pathways by binding to one of the two receptors: VEGF receptor-1 (VEGFR-1, previously termed fms-like tyrosine kinase-1 [Flt-1]) and VEGFR-2 (previously termed murine fetal liver kinase-1 [Flk-1] or kinase domain region [KDR] in humans) [52, 53]; as well as two co-receptors: neuropilin-1 (NRP1) and neuropilin-2 (NRP2). NRP1 enhanced VEGF signaling has been shown to be important for p38/MAPK activation, and is thus central to vessel branching [54, 55]. The expression of VEGFR3 is mainly restricted by the lymphatic endothelium in adult tissues. It binds VEGF-C and VEGF-D but not VEGF-A. And VEGFR3 is considered to control lymphangiogenesis [56]. VEGF mRNA can be firstly detected in fetal tissues at 16 weeks of gestation [57]. The expression of VEGF is particularly high in the lung, where it is essential in lung development and maintaining the structure of lung [58]. In human fetal lung, VEGF is localized in alveolar epithelial cells and myocytes, which suggested that VEGF acts a paracrine in modulating the activity of adjacent vascular endothelium [57]. In patients with BPD, VEGF also arises in Type II pneumocytes.

Roles of VEGF in BPD

In a comparative study of the causes between infants dying with BPD and non-pulmonary diseases, Bhatt found the former group had lower VEGF mRNA level and VEGF immunostaining than did the latter group [11]. Another study which investigated the expression of VEGF in tracheal aspirates revealed that preterm infants who developed BPD had lower VEGF levels during the early postnatal days than those without BPD. That suggests a prolonged and more severe respiratory distress [57]. Administration of anti-angiogenic agents to neonatal rats impairs both pulmonary angiogenesis and alveolarization [59-63]. Over-expression of proangiogenic factors, such as vascular endothelial growth factor (VEGF), alleviates the adverse effects of hyperoxia on Alveolarization [7, 42]. Inactivation of the VEGF- A gene in respiratory epithelium results in an absence of pulmonary capillaries, suggesting that the development of pulmonary capillary is in a VEGF-A dependent manner [64]. As a matter of fact, previous treatments for BPD with inhibitors of VEGF-A have shown that inhibition of angiogenesis seriously affected the formation of alveolar [59, 65].

Expression of VEGF-A is regulated by many factors including hypoxia (hypoxia-inducible factors-1α, HIF-1α), oncogene and tumor suppres-

Table 3. VEGF in BPD

Author	Model/human	Deal with	VEGF levels			Time of VEGF changing
			mRNA	Protein	Location	
Bhatt AJ et al. 2001 [11]	Human	Dead form BPD	↓	↓	Autopsy samples	P*65 ± 34 d
Tambunting F et al. 2005 [107]	Baboon	125 days gestation O_2	↓	↓	Lung specimens	P14 d
Balasubramaniam V et al. 2007 [12]	Neonatal mice	80% O_2 10 d		↓	Blood, lung, and bone marrow	P10 d
Been JV et al. 2010 [93]	Preterm infants	—		↓	BALF concentrat-ions	P0 d, P3 d
Grisafi D et al. 2013 [108]	Rats	60%O_2, 14 d	↓	↓	Lung sections	P14 d
Keenaghan M et al. 2013 [97]	Rats	10%, 21%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% FiO_2 for 2 h.		↓	Serum and lung	40% O_2 2 h
Firsova et al. 2014 [104]	Neonatal mice	95% O_2, 5 d		N#	Lung sections	p5, p28, and p56 d
Yang WC et al. 2015 [109]	Preterm infants	—		N	Cord blood	P0 d
Lajko M et al. 2016 [110]	Neonatal mice	75% o_2, P0-P14. room air 1 (P15), 7 (P21), or 14 days (P28)		↑	Retinal	p21 d
Kumar VH et al. 2016 [66]	Newborn mouse	85% O_2, P3-P15 Room air 15 weeks		↑	Lung sections	P15 w
Jin M et al. 2016 [111]	Newborn rats	21% or 85% O_2 7 d, room air 14 d	↓		Lung tissues	P7 d
Procianoy RS et al. 2016 [112]	Preterm neonates	72 h collected blood		↑	Peripheral blood	P72 h

Definition of abbreviations: P*: postnatal day; N#: normal.

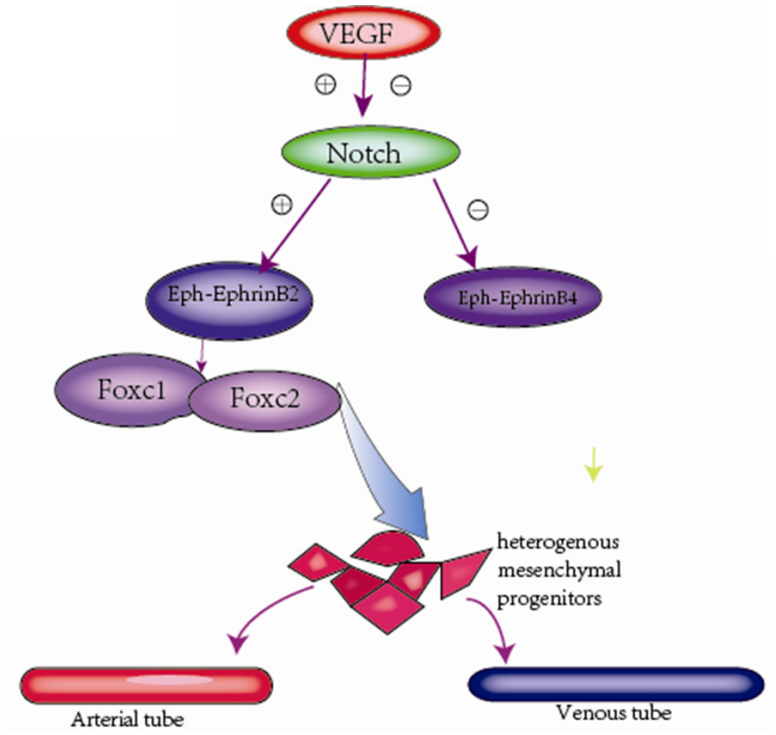

Figure 1. Angioblasts differentiate into endothelial cells which are prespecified to arterial or venous phenotypes by Notch signaling. Endothelial differencetitation: Arterial and venous specification. when Notch increase, the Arterial tube conforming, and, when nothdecrease, the venous tube conforming.

sor dysregulation, transcription factors (TGF-α, TGF-β), inflammatory mediators (IL-1α, IL-1β, IL-6, TNFα), and mechanical forces of shear stress [47]. Therefore, mechanical ventilation and hyperoxia will, theoretically, increase inflammatory factors [66] and shear stress, and consequentially, the level of VEGF. But most studies showed that expression of VEGF in histological sections of BPD patients or animal models decreased (**Table 3**). However, Tomanek's study on explanted embryonic quail hearts indicates that vascular formation can be enhanced by hypoxia (5-10% O_2) and inhibited by hyperoxia [67]. Nevertheless, why does not VEGF increase in BPD? We proposed three possible reasons according to previous reports: 1. Severe lung injury may render VEGF incapable of responding to the inflammatory stimuli. 2. An increased level of VEGF after birth is locally secreted, since VEGF acts as a mediator of paracrine. And when lungs were injured by postpartum ventilators, infections, and oxygen/nitrogen free radicals, locally increased VEGF do not well accelerate vascular development; 3. Hyperoxia (Postpartum oxygen or ventilator support) inhibited HIF-1α which can enhance the expression of VEGF [68].

Mechanisms of VEGF take part in vasculogenesis and angiogenesis

The formation of new blood vessels can be divided into two stages: vasculogenesis and angiogenesis [69]. Vasculogenesis starts from angioblasts or endothelial precursor cells which migrate and differentiate into local cues (growth factors, extracellular matrix), and further develop into vascular tubes, a process from nil to existence. Angiogenesis is the formation of new blood vessels from preexisting ones, which is a process from less to more [70]. However, VEGF is involved in many aspects of angiogenesis, including survival, proliferation, migration, tubulogenesis, remodeling and quiescence.

Differentiation of endothelial cells

Angioblasts are differentiated into endothelial cells (ECs). ECs develop into the cords and form a lumen, whose phenotype can be distinguished into artery or vein. Arterial and venous ECs possess the ability of identifying specific molecules [71, 72]. Components of the notch signaling pathway which is activated by VEGF are highly expressed in arteries and are deficient in veins. Thus, inhibition of the Notch signaling pathway causes loss of arterial markers and re-expression of specific genes in veins [72, 73]. The Notch signaling pathway also regulates the expression of members of Eph-Ephrin family and Ephrin-B2. Ephrin-B2 is increased in response to Notch, whereas its receptor EphB4 in venous ECs is repressed by Notch (**Figure 1**).

Angiogenesis

Angiogenesis (neovascularization) occurs through a series of steps which consist of angiogenic stimulus, sprouting, elongation and branching, formation of vessel lumen, anastomosis and finally stabilization [9]. ECs become motile and invasive and protrude filopodia in response to VEGF released by matrix metallo-

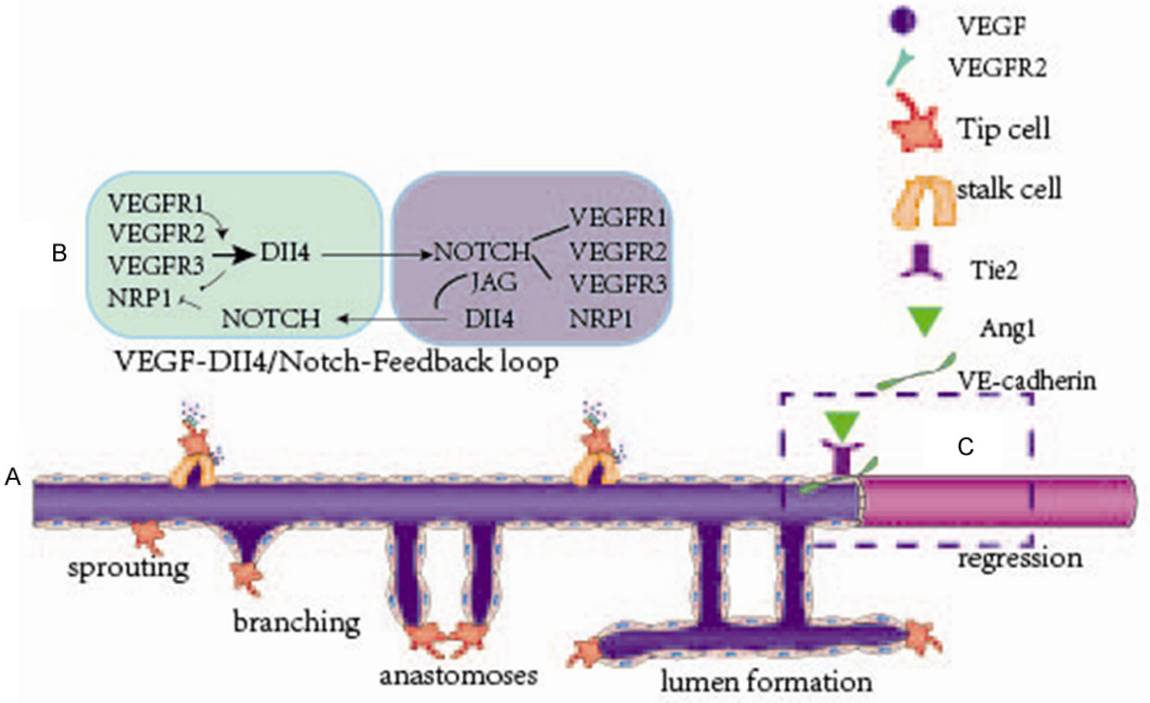

Figure 2. A: Steps of Angiogenesis; B: The feedback loop between VEGF and Notch; C: Ang1 activates Tie2 to stabilize vessels, promotes pericyte adhesion, and makes them leak.

proteinases (MMPs) [48], These so-called tip cells will sprout new ones; stalk cells seldom generate filopodia, but they establish a lumen and proliferate to support elongation of sprouts. Tip cells anastomose with cells from neighboring sprouts to set up vessel loops. Tip and stalk cells are affected by VEGF/Notch signaling [74] (**Figure 2B**). When blood begins to flow, the establishment of the basement membrane and the recruitment of mural cells stabilize new connections. The increase in oxygen and nutrient decreases the expression of VEGF and inactivates the sensors of endothelial oxygen with the blood perfusion, meanwhile the phenotype of endothelial behavior is shifted into a quiescent one (**Figure 2A**).

Maturation, stabilization, and quiescence of vessels

At the last stage of angiogenesis, the newly formed blood carries mural cells or pericytes to maintain stability of capillaries [75]. The role of pericytes in the function and angiogenesis of capillaries includes regulation of EC proliferation and migration, as well as production of basement membrane of capillary together with ECs [76]. Adherence junction molecules mediate cell-cell adhesion, cytoskeletal reorganization, and intracellular signal transduction. VE-cadherin is one key component of EC junctions. In the case with VEGFR2 compound, VE-cadherin keeps EC static through dephosphorylate VEGFR2 to further inhibit VEGF signaling. Different types of VE-cadherin-based adherence junctions establish stable or transitory interactions with the cytoskeletons which can either solidify EC adhesion or facilitate EC separation and movement. Angiopoietin-1 (ANG1), produced by mural cells, activates its endothelial receptor TIE2 [77, 78] and plays a very important role in stabilizing the structure of vessels, promoting adhesions of pericytes, and tightening endothelial junctions (**Figure 2C**).

Exploring the application of VEGF in BPD treatment

Current methods of treating BPD include caffeine [79], nutrients, vitamin A [80], vitamin D [81], glucocorticoids [82], antibiotics [19], mesenchymal stromal cells (MSCs) [27, 83, 84] and BMSCs in combination with erythropoietin [85]. The VEGF gene was successfully used to treat limb after ischemia [86]. In recent years, researchers tried to promote angiogenesis of

bone tissue and ischemic myocardium through the VEGF gene therapy, and have made some satisfying achievements [87]. The application of VEGF in the treatment of BPD has also been investigated. Kunig et al [13] observed two-day-old Sprague-Dawley rats that were placed into hyperoxia or room air (RA) for 12 days. At 14 days, rats respectively received daily treatment with recombinant human VEGF (rhVEGF)-165 or saline. And they found rhVEGF treatment during the period of recovery accelerated vessels growth and alveolarization after hyperoxic lung injury in neonatal rats. He found fetal lung explants from eNOS(-/-) mice decreased the formation of terminal lung buds, it was restored with rhVEGF treatment [14], a finding sililar to that of Seedorf's study. The postnatal intratracheal adenovirus-mediated VEGF gene therapy remarkably improves the survival, promotes the formation of lung capillaries, and preserves the development of alveolars in BPD model of irreversible lung injury [70]. To determine whether disruption of vascular endothelial growth factor receptor (VEGFR) signaling in the newborn has long-term effects on lung structure and function, Le Cras et al injected 1-day-old newborn rat pups with a single dose of Su-5416, a VEGFR inhibitor, or vehicle (controls). Lungs from infant (3-wk-old) and adult (3- to 4-mo-old) rats treated with Su-5416 showed reductions in arterial density (82 and 31%, respectively) and alveolar counts (45 and 29%) compared with the controls. Treatment for neonatals with Su-5416 increased right ventricle weight to body wt ratios (4.2-fold and 2.0-fold) and pulmonary arterial wall thickness measurements (2.7-fold and 1.6-fold) in infant and adult rats, respectively, indicating marked pulmonary hypertension. We conclude that treatment of newborn rats with the VEGFR inhibitor Su-5416 impairs the pulmonary vascular growth and postnatal alveolarization and causes pulmonary hypertension and that these are long-term effects lasting well into adulthood [88]. As the expression of HLA class I and II molecules are very low, MSCs cannot trigger an immune response once administered to animals or humans in an allogeneic MSCs [84, 89]. Moreover, MSCs have been shown to effectively ameliorate experimental BPD when administered in a preventive or therapeutic way [90, 91]. Chang studied intratracheal MSC transplantation which was performed in 9 preterm infants, with a mean gestational age of 25.3 ± 0.9 weeks and a mean birth weight of 793 ± 127 g, at a mean of 10.4 ± 2.6 days after birth. The first 3 patients were given a low dose (1×10^7 cells/kg) of cells, and the other 6 were given a high dose (2×10^7 cells/kg). Having compared their adverse outcomes, including BPD severity, with those of the historical case-matched comparison group, they conclude that intratracheal transplantation of allogeneic hUCB-derived MSCs in preterm infants is safe and feasible, and warrants a larger and controlled phase II study [92]. Several phase 1 and phase 2 trials are in progress (NCT02443961, NCT02381366, NCT01828957) [84]. MSCs, derived from bone marrow stroma with the ability of self-renewal, can be divided into mesodermal stem cells, and a variety of cells such as endothelial cells and endothelial progenitor cells. These cells can conjugate with VEGF and better to promote the formation of pulmonary vessels.

The timing of using VEGF to treat BPD

We found that infants diagnosed with BPD after birth did not have lower levels of VEGF in umbilical cord blood than infants without BPD (**Table 3**). Been's view [93] was different from other researcher's. He believes that VEGF in newborns with BPD decreased in the first day after birth. The possible reason is that the patients in his study have basic characteristics different from those in other studies, a lower gestational age of patients, for example. The results would be inconsistent. Higher lavage VEGF levels on days 1 and 3 were also correlated with a lower gestational age after birth [94]. The accumulation of VEGF may aggravate the body injury. Zeng's study found that over-expression of VEGF in fetal murine lungs not only enhanced pulmonary vasculogenesis but also resulted in an abnormal alveolar development [95], It is not necessary to administer VEGF in the first day after birth. We summarized from previous studies that with the increase of the oxygen concentration, VEGF decreases sooner (**Table 3**). In the condition of moderate oxygen (60%) [12, 96], VEGF in mice decreases on the 14th day after birth. Here are two key points: Firstly, there exist difference between the models of mouse with BPD and humans with BPD, We still do not monitor the changes of VEGF in infants with BPD before they died. Whereas the autopsy materials from non-survivors with BPD present one avenue for the exploration of pathogen-

ic mechanisms at play in the lungs of affected patients. These materials are increasingly rare and difficult to obtain, because the survival rate of BPD patients has steadily increased over time. Both mice and rats are delivered at term in the saccular stage of lung development, and this fact is often used to justify the superiority of mice and rats as model animals for BPD, since preterm infants that develop BPD are also delivered in the saccular stage of lung development [1]; Secondly, the time of decline of VEGF is one key point in establishing the model of mouse with BPD. The amount of VEGF in lung sections still cannot be continuously monitored; whether VEGF is declined or not before BPD needs further researches. Keenaghan used FiO_2 exposed rats for 2 hours, and found VEGF decreased on 40% in 2 h [97].

Conclusion

VEGF signaling pathway acts as one key mechanism in the pathology of BPD, and treatment for infants with BPD by VEGF improves the outcome. We summarized that treatment of VEGF for infants with BPD before preterm infants 14 days after birth may effectively prevent BPD, but the exact time of treating for BPD still needs further researching.

Disclosure of conflict of interest

None.

Address correspondence to: Zhichun Feng, Affiliated Bayi Children's Hospital, Clinical Medical College in PLA Army General Hospital, Southern Medical University, Beijing, China. E-mail: zhjfengzc@126.com

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