Molecular Biology Reports
https://doi.org/10.1007/s11033-020-05831-7

SHORT COMMUNICATION

# Intussusceptive angiogenesis in Covid-19: hypothesis on the significance and focus on the possible role of FGF2

**Simone Meini**[1] · **Tommaso Giani**[2] · **Carlo Tascini**[3]

Received: 19 July 2020 / Revised: 1 September 2020 / Accepted: 7 September 2020


**Abstract**
The interest on the role of angiogenesis in the pathogenesis and progression of human interstitial lung diseases is growing, with conventional sprouting (SA) and non-sprouting intussusceptive angiogenesis (IA) being differently represented in specific pulmonary injury patterns. The role of viruses as key regulators of angiogenesis is known for several years. A significantly enhanced amount of new vessel growth, through a mechanism of IA, has been reported in lungs of patients who died from Covid-19; among the angiogenesis-related genes, fibroblast growth factor 2 (FGF2) was found to be upregulated. These findings are intriguing. FGF2 plays a role in some viral infections: the upregulation is involved in the MERS-CoV-induced strong apoptotic response crucial for its highly lytic replication cycle in lung cells, whereas FGF2 is protective against the acute lung injury induced by H1N1 influenza virus, improving the lung wet-to-dry weight ratio. FGF2 plays a role also in regulating IA, acting on pericytes (crucial for the formation of intraluminal pillars), and endothelium, and FGF2-induced angiogenesis may be promoted by inflammation and hypoxia. IA is a faster and probably more efficient process than SA, able to modulate vascular remodeling through pruning of redundant or inefficient blood vessels. We can speculate that IA might have the function of restoring a functional vascular plexus consequently to extensive endothelialitis and alveolar capillary micro-thrombosis observed in Covid-19. Anti-Vascular endothelial growth factor (anti-VEGF) strategies are currently investigated for treatment of severe and critically ill Covid-19 patients, but also FGF2, and its expression and/or signaling, might represent a promising target.

**Keywords** Angiogenesis · Intussusceptive · FGF2 · VEGF · Covid-19 · Virus

## Introduction

The interest on the role of angiogenesis in the pathogenesis and progression of human interstitial lung diseases is growing, with conventional sprouting (SA) and non-sprouting intussusceptive angiogenesis (IA) being differently represented in specific pulmonary injury patterns [1]. It has been hypothesized that microvascular alterations represent one of the first steps in the pathogenesis of idiopathic pulmonary fibrosis [1, 2], and morphogenetic phenomena of plexiform vasculopathy with pronounced IA have been described also in chronic thromboembolic pulmonary hypertension [3]. The interest on this argument, largely still to be deciphered, recently went to Covid-19, a respiratory illness often characterized by a picture of interstitial pneumonia able to progress towards a life-threatening acute respiratory distress syndrome and to further evolve in pulmonary fibrosis.

In this short paper we address the role of angiogenesis in the pathogenesis of Covid-19, briefly reviewing what is to date known on the interplay between viruses and angiogenic processes and speculating on the mediators involved.

✉ Simone Meini
simonemeini2@gmail.com

Tommaso Giani
tommaso.giani@unifi.it

Carlo Tascini
c.tascini@gmail.com

[1] Internal Medicine Unit, Santa Maria Annunziata Hospital, Florence, Italy

[2] Microbiology and Virology Unit, Careggi Hospital, University of Florence, Florence, Italy

[3] Infectious Diseases Clinic, Santa Maria Misericordia Hospital, University of Udine, Udine, Italy

Published online: 12 September 2020

## Angiogenesis and viral infections

Angiogenesis is the physiological process through which new blood vessels form from pre-existing vessels; in adults, neovascularization occurs during wound healing and female reproductive cycle, thus representing a well-regulated process. Unregulated angiogenesis can be observed in pathological situations, and the role of viruses as key regulators of angiogenesis is known for several years. Viruses may regulate this process either directly or indirectly, by the activation of vessels through endothelial cell tropism and by the production of chemokines and/or growth factors, inducing an inflammatory response and creating a pro-angiogenic microenvironment [4].

Cytomegalovirus (CMV) infection may have detrimental consequences dysregulating the normal angiogenic process, being able to promote and enhance all stages of angiogenesis, inducing the activation and proliferation of endothelial cells; moreover, infected cells produce vascular-endothelial growth factor (VEGF) and other angiogenic factors, such as angiopoietins, fibroblast-growth factor (FGF) and platelet-derived growth factor (PDGF) [5, 6]. Emerging evidence from human and mouse models indicates that CMV can promote tumor angiogenesis and proliferation [6], and also Epstein-Barr virus (EBV) and Kaposi's sarcoma-associated human herpes virus 8 (HHV8) can critically contribute to carcinogenesis inducing both angiogenesis and lymphangiogenesis [7]. Hepatitis C virus (HCV) proved to play a crucial role in the regulation of angiogenesis in hepatocellular carcinoma progression, inducing the production of VEGF; however, little is known about the molecular mechanisms which are responsible for the modulation of HCV-promoted hepatic angiogenesis, and also angiopoietin-2 is thought to play an important role [8]. The Orf virus, a zoonotic parapoxvirus that may be transmitted to humans, rarely provokes an extensive vasculo-endothelial proliferation through the early expression of a VEGF-like viral gene product [9]. Moreover, despite the infection of fibroblasts by parvovirus B19 has been suggested to have anti-angiogenic effects in vitro [10], it has been recently reported [11] that parvovirus B19-related induction of myocarditis caused a distinct formation of new blood vessels around inflammatory sites both by SA and IA. An anti-angiogenic effect has been instead observed in other viral models: Zika virus (ZIKV), besides neurogenesis, also seems impair angiogenesis during murine embryonic development [12], and Dengue virus infection proved to inhibit SA in vitro [13].

Very recently, Ackermann et al. [14] reported a significantly enhanced new vessel growth, predominantly through a mechanism of IA, in lungs of patients who died from Covid-19 rather than influenza A(H1N1), and the degree of IA in Covid-19 was found to increase significantly and progressively over time during hospitalization. These findings were unexpected, but it is known that IA is a faster and probably more efficient process than SA, able to modulate the vascular remodeling through pruning of redundant or inefficient blood-vessels [15]. IA could therefore represent an attempt to restore a functional vascular plexus consequently to the extensive endothelialitis and alveolar capillary microthrombosis observed in Covid-19: alveolar capillary microthrombi are indeed 9 times as prevalent in patients with Covid-19 as in patients with influenza [14].

### Molecular control of intussusceptive angiogenesis and the interplay with viral infections

To date, scarce data are available on the molecular control of IA [15], but, from a pathogenetic point of view, the occurrence of phenomena of IA more pronounced in lungs of patients with severe Covid-19 rather than with influenza leads us to hypothesize in the two viral models a different regulation of the mediators involved.

Apart from VEGF and angiopoietins, it is known that (basic) fibroblast growth factor 2 (FGF2) plays a role in regulating IA, acting on endothelium and on pericytes, which are crucial for the formation of intraluminal pillars [15, 16]. FGF2 exerts its pro-angiogenic activity interacting with various cell surface receptors, including the tyrosine kinase receptors, heparan-sulfate proteoglycans and integrins [16]. FGF2-dependent angiogenesis may be promoted by hypoxia and inflammation (neoangiogenesis and inflammation are closely related processes), FGF2 induces vascular permeability and vasodilation [16], and the production of this factor from endothelium can be triggered by nitric oxide [16]: the formation of intussusceptive pillars is indeed preceded by vasodilation [1]. FGF2 has been implicated in the pathogenesis of Kaposi's sarcoma, an angioproliferative disorder that requires infection with the HHV 8 [17]; moreover, FGF2 represents a pro-angiogenic host factor during infections due to human papilloma virus (HPV) and human T-cell lymphotropic virus type 1 (HTLV-1) [4].

Among angiogenesis-related genes, that one encoding FGF2 was found to be upregulated in lungs from patients who died from Covid-19, but not in those with influenza A(H1N1) [14]. These findings are intriguing, because FGF2 has shown to play a role in several viral models. Middle East respiratory syndrome coronavirus (MERS-CoV) induces a strong apoptotic response in lung cells through upregulation of the FGF2 expression, and apoptosis seems crucial for completion of the highly lytic MERS-CoV replication cycle [18]. In influenza A(H1N1) infection, FGF2 resulted instead protective against acute lung injury, and FGF2 depletion or knockout-mice showed significantly decreased survival rates

and significantly increased wet-to-dry ratios of the lung tissue [19]. Massive interstitial edema and much higher weight were indeed reported in lungs from patients who died from influenza (where the FGF2-upregulation was not detected) rather than from Covid-19 [14].

In addition, Ackermann et al. [14] reported the upregulation of gene encoding VEGF-A in lungs from patients who died both from Covid-19 and influenza A(H1N1), while the upregulation of VEGF-C (the major effector of lymphangiogenesis) was observed only in Covid-19. It is known that VEGF plays a relevant role in IA. Gianni-Barrera et al. [20] observed that VEGF over-expression, at the doses required to induce therapeutic benefit, induces vascular growth in skeletal muscle by IA rather than SA, and it has been suggested [21] that the transition between normal and aberrant angiogenesis depends on the balance of the relative stimulation of VEGF and PDGF-BB signaling: pericytes, recruited by PDGF-BB, play complex regulatory functions, promoting vascular stabilization and preventing aberrant angiogenesis by excessive VEGF.

Interestingly, the reduction of VEGF-A levels led to vascular tree regression by intussusceptive vascular pruning (an important facet of IA) [22]: this observation seems suggest that VEGF inhibition might be associated with vessel normalization by modulating IA. The role of Bevacizumab (an anti-VEGF monoclonal antibody) in severe or critically severe patients with Covid-19 pneumonia is currently investigated in a randomized clinical trial (RCT) (ClinicalTrials.gov Identifier NCT04305106).

## Could the FGF2 be a target against SARS-CoV-2 infection?

The hyper-expression of FGF2 could be implicated in the pathogenesis of coronavirus infections [18], as well as in the prominent morphogenetic phenomena of IA reported in Covid-19 [14], but we don't know whether these phenomena should be considered adaptive and beneficial for the host, and we should modulate them in a positive way, or not.

Chloroquine, a drug that has been widely used in Covid-19 patients with controversial results, showed to potentiate the FGF2-induced production of tissue-plasminogen activator (t-PA) by endothelial cells, and to modulate their activity in a complex fashion, inhibiting the FGF2-induced mitogenic activity in a dose-dependent manner [23].

A potent mitogenic signaling resulted instead from heparin-mediated trans-dimerization of FGF2 [24]: both unfractionated and low-molecular-weight heparins are currently investigated in several RCTs as therapeutic options for Covid-19.

The full significance of findings on the FGF2-related pathway in Covid-19 still remains to be understood.

## Conclusions

Prominent morphogenetic phenomena of IA have been observed in lungs from patients who died from Covid-19, and a distinct pattern of angiogenesis-associated genes expression has been described [14]. The clinical implications of these findings require further research, and our considerations are meant solely as a stimulus for the generation of novel hypotheses about the pathogenetic mechanisms underlying SARS-CoV-2 infection. A thorough comprehension of Covid-19 pathogenesis is crucial to individuate effective therapeutic approaches. Anti-VEGF strategies are currently investigated for the treatment of severe and critically ill Covid-19 patients, but also FGF2, and its expression and/or signaling, might represent a promising target.

### Compliance with ethical standards

**Conflict of interest** No potential conflict of interest relevant to this manuscript was reported.

## References


1. Ackermann M, Stark H, Neubert L et al (2020) Morphomolecular motifs of pulmonary neoangiogenesis in interstitial lung diseases. Eur Respir J 55(3):1900933. https://doi.org/10.1183/13993003.00933-2019
2. Puxeddu E, Cavalli F, Pezzuto G et al (2017) Impact of pulmonary vascular volume on mortality in IPF: is it time to reconsider the role of vasculature in disease pathogenesis and progression? Eur Respir J 49:1602345
3. Ackermann M, Gaumann A, Mentzer SJ et al (2017) Plexiform vasculopathy in chronic thromboembolic pulmonary hypertension. Am J Respir Crit Care Med 196:e48–e51
4. Vrancken K, Vervaeke P, Balzarini J, Liekens S (2011) Viruses as key regulators of angiogenesis. Rev Med Virol 21(3):181–200
5. Caposio P, Orloff SL, Streblow DN (2011) The role of cytomegalovirus in angiogenesis. Virus Res 157(2):204–211
6. Cobbs C (2019) Cytomegalovirus is a tumor-associated virus: armed and dangerous. Curr Opin Virol 39:49–59
7. Sakakibara S, Tosato G (2009) Regulation of angiogenesis in malignancies associated with Epstein-Barr virus and Kaposi's sarcoma-associated herpes virus. Future Microbiol 4(7):903–917
8. Hassan M, Selimovic D, El-Khattouti A et al (2014) Hepatitis C virus-mediated angiogenesis: molecular mechanisms and therapeutic strategies. World J Gastroenterol 20(42):15467–15475
9. Aşiran Serdar Z, Yaşar Ş, Demirkesen C, Aktaş Karabay E (2018) Poxvirus-induced vascular angiogenesis mimicking pyogenic granuloma. Am J Dermatopathol 40(9):e126–e129
10. Arvia R, Margheri F, Stincarelli MA et al (2020) Parvovirus B19 activates in vitro normal human dermal fibroblasts: a possible implication in skin fibrosis and systemic sclerosis. Rheumatology. https://doi.org/10.1093/rheumatology/keaa230
11. Ackermann M, Wagner WL, Rellecke P, Akhyari P, Boeken U, Reinecke P (2020) Parvovirus B19-induced angiogenesis in fulminant myocarditis. Eur Heart J 41(12):1309

12. Garcez PP, Stolp HB, Sravanam S et al (2018) Zika virus impairs the development of blood vessels in a mouse model of congenital infection. Sci Rep 8(1):12774
13. Basu A, Jain P, Sarkar P et al (2011) Dengue virus infection of SK Hep1 cells: inhibition of in vitro angiogenesis and altered cytomorphology by expressed viral envelope glycoprotein. FEMS Immunol Med Microbiol 62(2):140–147
14. Ackermann M, Verleden SE, Kuehnel M et al (2020) Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in Covid-19. N Engl J Med. doi:https://doi.org/10.1056/NEJMoa2015432
15. De Spiegelaere W, Casteleyn C, Van den Broeck W et al (2012) Intussusceptive angiogenesis: a biologically relevant form of angiogenesis. Vasc Res 49(5):390–404
16. Presta M, Andrés G, Leali D, Dell'Era P, Ronca R (2009) Inflammatory cells and chemokines sustain FGF2-induced angiogenesis. Eur Cytokine Netw 20(2):39–50
17. Ensoli B, Gendelman R, Markham P et al (1994) Synergy between basic fibroblast growth factor and HIV-1 Tat protein in induction of Kaposi's sarcoma. Nature 371:674–680
18. Yeung ML, Yao Y, Jia L et al (2016) MERS coronavirus induces apoptosis in kidney and lung by upregulating Smad7 and FGF2. Nat Microbiol 1(3):16004
19. Wang K, Lai C, Li T et al (2018) Basic fibroblast growth factor protects against influenza A virus-induced acute lung injury by recruiting neutrophils. J Mol Cell Biol 10(6):573–585
20. Gianni-Barrera R, Trani M, Fontanellaz C et al (2013) VEGF over-expression in skeletal muscle induces angiogenesis by intussusception rather than sprouting. Angiogenesis 16(1):123–136
21. Gianni-Barrera R, Bartolomeo M, Vollmar B, Djonov V, Banfi A (2014) Split for the cure: VEGF, PDGF-BB and intussusception in therapeutic angiogenesis. Biochem Soc Trans 42(6):1637–1642
22. Hlushchuk R, Ehrbar M, Reichmuth P et al (2011) Decrease in VEGF expression induces intussusceptive vascular pruning. Arterioscler Thromb Vasc Biol 31(12):2836–2844
23. Inyang AL, Bikfalvi A, Lu H, Tobelem G (1990) Chloroquine's modulation of endothelial cell activity induced with basic fibroblast growth factor and human serum: effect on mitogenesis, protease production and cell migration. Cell Biol Int Rep 14(1):35–46
24. Goodger SJ, Robinson CJ, Murphy KJ et al (2008) Evidence that heparin saccharides promote FGF2 mitogenesis through two distinct mechanisms. J Biol Chem 283(19):13001–13008