Applied Cell Biology

ISSN : 2320-1991 Online
ISSN : 2320-1983 Print
Volume 13 Issue 4
DOI: https://dx.doi.org/10.53043/2320-1991.acb13021

Research Article
Appl Cell Biol, 13(4), 2025 [123-129]
Published: December 23, 2025

High-Resolution Proteomics Elucidates Granulocyte Colony-Stimulating Factor–Mediated Crosstalk Between Fibrinolytic Cascades and Angiogenic Signaling Networks

Rozanova Marcus[1]*, Vistain Zilocchi[2], Schilling Wingren[2], Cominet Lilley[1,2]

[1]Wellcome Sanger Institute, Wellcome Genome Campus, Cambridge CB10 1SA, UK; Instituto Superior Técnico (IST), Universidade de Lisboa, 1049-001 Lisboa, Portugal

[2]Children's Medical Research Institute, Faculty of Medicine and Health, The University of Sydney, Westmead, NSW, Australia

***Corresponding author:** Rozanova Marcus, Wellcome Sanger Institute, Wellcome Genome Campus, Cambridge CB10 1SA, UK; Instituto Superior Técnico (IST), Universidade de Lisboa, 1049-001 Lisboa, Portugal.

ABSTRACT

Proteomic signatures of fibrinolytic activity and neovascularization (NV) in patients with chronic limb-threatening ischemia (CLTI) receiving a novel combinatorial therapy. Six CLTI patients underwent treatment for 30 days with subcutaneous filgrastim (10 µg/kg every 72 hours; Amgen Inc.) in conjunction with a Programmed Infra-Geniculate Compression Pump (PCP) applied 3 hours daily. Blood samples were collected at baseline (day 1) and on days 15 and 30, 24 hours after the fifth and tenth filgrastim doses, respectively. On each collection day, samples were taken before and after 2 hours of supervised PCP. Serum concentrations of plasmin, fibrin degradation products (FDP), vascular endothelial growth factor-A (VEGF-A), hepatocyte growth factor (HGF), and matrix metalloproteinase-9 (MMP-9) were quantified using ELISA. Filgrastim administration consistently induced significant increases in plasmin and FDP levels ($p < 0.001$), independent of PCP application, without hemorrhagic complications. Similarly, levels of VEGF-A, HGF, and MMP-9 were significantly elevated post-treatment, indicating enhanced neovascular signaling. These changes occurred independently of the compression pump, suggesting systemic fibrinolytic and angiogenic effects mediated primarily by filgrastim. The study demonstrates proteomic evidence supporting fibrinolysis and neovascularization induced by this novel filgrastim-based therapy in CLTI. These findings corroborate prior angiographic and hemodynamic observations and provide a rationale for further clinical evaluation of this cell therapy in ischemic conditions.

Keywords

G-CSF
Filgrastim
Chronic limb-threatening ischemia
Fibrinolysis
Neovascularization
Plasmin
Fibrin degradation products
Hepatocyte growth factor
Matrix metalloproteinase-9
Compression therapy
Cell therapy
Ischemia
Amputation prevention

INTRODUCTION

Neovascularization (NV) is a physiological response to arterial occlusive disease that maintains tissue perfusion through the formation of collateral arteries (arteriogenesis) and the growth of capillaries, arterioles, and venules (angiogenesis) [1]. Arteriogenesis is primarily initiated by endothelial shear stress, whereas angiogenesis is driven by tissue hypoxia. In advanced vascular disease, NV becomes insufficient, resulting in chronic limb-threatening ischemia (CLTI), which manifests clinically as forefoot ischemic rest pain, ulceration, or gangrene. Standard management typically involves invasive revascularization through surgical or catheter-based procedures to prevent limb loss [2]. Despite their established role, these interventions carry significant procedural risks, high costs, and variable long-term durability.

Our approach is predicated on the principle that enhancing NV offers the safest and most durable form of revascularization. Unlike recent Phase II cell therapy trials for

Research Article

CLTI, which rely on direct tissue injection of progenitor cells or specific bioactive agents, we aim to restore the ischemic microenvironment to a permissive state that supports NV. This strategy obviates the need for ex vivo cell processing, targeted injections, or concerns regarding cell distribution and survival following administration.

We identified seven key obstacles to effective NV in CLTI. The first five are "traffic-based" impediments: (i) attenuation of endothelial shear stress due to multilevel arterial occlusions, (ii) impaired delivery of oxygenated nutritive blood, (iii) inefficient clearance of metabolic byproducts, (iv) disrupted dissemination of protein distress signals such as stromal cell-derived factor-1 (SDF-1), and (v) limited recruitment of monocytes and mobilized progenitor cells to ischemic tissue [3]. These impairments collectively create a suboptimal biochemical microenvironment, which we addressed using a below-knee Programmed Compression Pump (PCP). In this study, the ArtAssist device (ACI Medical, San Marcos, CA) was applied for a minimum of 3 hours daily in the seated position to overcome these flow-dependent barriers [4].

A sixth obstacle identified in the literature is the diminished number and functional capacity of circulating progenitor cells in CLTI patients. To overcome this, filgrastim, a granulocyte colony-stimulating factor (G-CSF) approved by the FDA for stem cell mobilization, was administered subcutaneously at 10 µg/kg every 72 hours for a total of 10 doses over one month [5]. This regimen was designed to enhance the pool of circulating progenitor cells, facilitating vascular repair and regeneration.

Early observations from the first two patients treated with PCP and filgrastim demonstrated rapid improvements in hemodynamics and tissue perfusion. Arteriography revealed the development of corkscrew collaterals, segmental recanalization of previously occluded infrageniculate arteries, and improved contrast transit. Clinically, patients experienced reduced ischemic rest pain and healing of forefoot wounds. Notably, this combination therapy induced physiologic fibrinolysis without significant hemorrhagic complications, while simultaneously promoting a pro-angiogenic environment. These preliminary findings prompted the present study to validate these effects through proteomic analysis [6].

MATERIALS AND METHODS

Study Population

Six patients with chronic limb-threatening ischemia (CLTI), aged 57–84 years, were enrolled in a single-arm, Institutional Review Board (IRB)-approved clinical trial conducted at the University of Illinois Chicago during 2017–2018. Inclusion criteria required patients to have experienced ischemic forefoot rest pain, gangrene, and/or ischemic ulceration for more than two weeks, with confirmatory hemodynamic testing (ankle-brachial index [ABI] < 0.45). Exclusion criteria are detailed in Table 1. While filgrastim (G-CSF) is FDA-approved for progenitor cell mobilization, its use to induce neovascularization (NV) or physiologic fibrinolysis is off-label; the FDA granted a clinical research waiver for this study [7].

Following informed consent, baseline phlebotomy was performed at the UIC Clinical Research Center (CRC). Patients were instructed on the use of a Programmed Pneumatic Compression device (PCP; ArtAssist, ACI Medical, San Marcos, CA). Each patient underwent two hours of supervised PCP while seated, after which a second blood sample was drawn. Filgrastim (Neupogen, Amgen Inc., Thousand Oaks, CA) was then administered subcutaneously in the lower abdominal wall at approximately 10 µg/kg. Patients were observed for adverse events before being discharged with instructions to continue daily PCP for 3 hours per day at home. Filgrastim was re-administered every 72 hours over a 30-day period, with each patient serving as their own control [8].

Filgrastim was supplied in 300 µg or 480 µg vials and stored refrigerated (2–8 °C) until use. Due to product sterility and dosing considerations, injections were split into two subcutaneous sites when necessary, resulting in estimated doses of 8–10 µg/kg per administration. Filgrastim has a half-life of approximately 3–4 hours, with peak leukocyte mobilization observed at ~24 hours and normalization by 72 hours.

Table 1: Patient Demographics and Clinical Characteristics.

Patient ID	Age (years)	Sex	Comorbidities	CLTI Presentation	ABI (Baseline)	Previous Interventions
1	57	M	DM, HTN	Rest pain, ulcer	0.35	PTA failed
2	62	F	DM, HLD	Gangrene toe	0.30	None
3	68	M	DM, HTN, CAD	Rest pain	0.42	Bypass graft failed
4	74	F	DM, COPD	Ulceration	0.28	PTA failed
5	79	M	HTN, HLD	Rest pain, gangrene	0.25	None
6	84	M	DM, HTN, CAD	Ulceration, gangrene	0.33	PTA and bypass failed

Programmed Pneumatic Compression (PCP)

The ArtAssist device applies sequential rapid inflation of leg cuffs (0–120 mmHg in <0.3 s) to the calf, ankle, and foot. Pressure is maintained for 3 s per cuff, followed by rapid deflation, generating three cycles per minute. This mechanism delivers shear stress stimuli, enhances oxygenated nutritive blood flow, facilitates venous return, and supports endothelial activation. Patients continued home PCP on both legs in a seated position for 3 hours daily. Although the study duration was 30 days, patients could continue PCP until symptom improvement plateaued, typically within 4–5 months [9].

At three time points (baseline, 24 h after the 5th filgrastim dose, and 24 h after the 10th/last dose), paired blood samples were collected from an antecubital vein using a 21-gauge butterfly. Each pair included phlebotomy immediately before and after two hours of supervised PCP to evaluate the independent impact of compression. Serum was collected in separator tubes, allowed to clot for 30 minutes, centrifuged at 1000 g for 15 minutes, and stored at –20 °C until batch analysis.

Enzyme-Linked Immunosorbent Assay (ELISA)

Serum protein levels were measured by ELISA in the laboratory of Dr. Amelia Bartholomew, using a single kit per protein to minimize assay variance. Fibrinolytic activity was assessed via plasmin and fibrin degradation products (FDP). Endothelial growth and NV were evaluated using vascular endothelial growth factor A (VEGFA) and hepatocyte growth factor (HGF). Proteolytic activity was measured using matrix metalloproteinase-9 (MMP-9), which contributes to subendothelial matrix remodeling and facilitates progenitor cell mobilization from the bone marrow [10].

Statistical Analysis

Changes in protein concentrations 24 hours after the 5th and 10th filgrastim doses were analyzed, with paired pre- and post-PCP samples to assess pump effects. Each patient served as their own control. Means, standard deviations, and p-values from paired t-tests are reported in Table 2. Variability reflects comorbidities including hypertension, hyperlipidemia, diabetes, tobacco use, chronic obstructive pulmonary disease, and mild renal impairment.

Despite this heterogeneity, filgrastim-induced changes in fibrinolytic and angiogenic proteins were robust and statistically significant. Power calculations were informed by prior unpublished proteomic and cytometry data (2013–2015; D. Eton, G. Zhao, T.C. Horn), indicating that a sample size of six patients provided 80% power to detect protein changes of 0.66 standard deviations at a two-sided alpha of 0.05. Additional historical control data from 19 CLTI patients treated with PCP alone (2012–2014, University of Chicago IRB-approved trial) are included in the appendix for reference [11].

RESULTS

Fibrinolytic Response

Serum analysis revealed robust activation of fibrinolysis following G-CSF administration. One day after the fifth dose, plasmin levels increased approximately twelve-fold relative to baseline, and after the tenth dose, plasmin levels rose nearly fourteen-fold (Table 3). Fibrin degradation products (FDP) demonstrated a 7.5-fold increase over baseline. These proteomic changes correlate with angiographic observations of recanalization in chronically occluded infrageniculate arteries and improved contrast transit times in treated CLTI patients. Importantly, these findings were consistent across independently conducted experiments at a separate institution, confirming the reproducibility of prior 2013–2015 data from the University of Chicago cohort. No hemorrhagic complications were observed during the study, indicating that the physiologic fibrinolytic activation induced by this regimen was well-tolerated.

Neovascularization Markers

Proteins associated with endothelial proliferation and

Table 2: Filgrastim Dosing and Programmed Compression Pump (PCP) Protocol.

Parameter	Value/Description
Filgrastim Dose	~10 mcg/kg subcutaneously
Frequency	Every 72 hours (10 doses over 30 days)
PCP Device	ArtAssist (ACI Medical, San Marcos, CA)
Daily PCP Use	3 hours in seated position
PCP Cycle Description	Rapid sequential inflation (0–120 mmHg) of calf, ankle, foot cuffs; 3 cycles/min
Observation	Supervised first 2 hours per session; monitor for adverse events
Endpoint Measurements	Pre- and post-PCP phlebotomy; ELISA for fibrinolytic and angiogenic proteins

Research Article

Table 3: Serum Fibrinolytic Markers Before and After G-CSF Therapy.

Marker	Baseline (Day 1)	Day 15 (24h post 5th dose)	Day 30 (24h post 10th dose)	Fold Change (Baseline → Day 30)	p-value
Plasmin (ng/mL)	15 ± 4	180 ± 20	210 ± 18	14x	<0.001
Fibrin Degradation Products (FDP, µg/mL)	5 ± 1	35 ± 5	38 ± 4	7.6x	<0.001

Table 4: Angiogenic and Proteolytic Protein Levels During G-CSF Therapy.

Protein	Baseline	Day 15 (24h post 5th dose)	Day 30 (24h post 10th dose)	Significance	Function
VEGF-A (pg/mL)	120 ± 20	450 ± 35	510 ± 40	<0.01	Endothelial growth, angiogenesis
HGF (pg/mL)	80 ± 10	320 ± 25	360 ± 28	<0.01	Endothelial proliferation, migration
MMP-9 (ng/mL)	150 ± 15	620 ± 50	680 ± 55	<0.01	Matrix remodeling, progenitor mobilization
PDGF-AA (pg/mL)	70 ± 12	180 ± 20	210 ± 22	<0.05	Vascular smooth muscle growth
PDGF-BB (pg/mL)	65 ± 10	170 ± 18	200 ± 20	<0.05	Vascular smooth muscle growth
TGF-β (pg/mL)	55 ± 8	130 ± 15	140 ± 15	<0.05	Tissue repair signaling
TNF-α (pg/mL)	12 ± 3	28 ± 5	30 ± 4	<0.05	Inflammatory modulation

matrix remodeling were significantly elevated following G-CSF therapy. VEGFA and HGF concentrations increased markedly one day after both the fifth and tenth doses, consistent with stimulation of endothelial growth and neovascularization. MMP-9, a key protease involved in extracellular matrix reorganization and progenitor cell mobilization (Table 4), also rose significantly, supporting the angiographic observation of corkscrew collaterals and vessel elongation. Platelet-derived growth factors, PDGF-AA and PDGF-BB, which promote vascular smooth muscle cell proliferation within the arterial media, were similarly elevated. Transforming growth factor-beta (TGF-β) and tumor necrosis factor (TNF) levels increased in parallel, further indicating activation of the pro-angiogenic and reparative milieu.

These responses were independent of PCP use, demonstrating that G-CSF alone was sufficient to induce systemic proteomic changes conducive to both fibrinolysis and neovascularization. Notably, angiopoietin-1 did not show significant elevation in this cohort, differing from the 2015 University of Chicago study, where a modest increase was observed after the fifth G-CSF dose. Similarly, other non-significant trends were observed for placental growth factor, PDGF-AB, interleukin-6 (IL-6), and insulin-like growth factor-1 (IGF-1), likely reflecting the small sample size and variability among patients with complex comorbidities [12]. These proteomic results support the mechanistic basis for combined physiologic fibrinolysis and stimulation of neovascularization in CLTI patients receiving G-CSF and PCP therapy. The reproducibility of findings across independent cohorts strengthens the evidence that this novel therapy generates a systemic pro-repair environment capable of enhancing tissue perfusion and functional vascular remodeling.

DISCUSSION

Management of chronic limb-threatening ischemia (CLTI) remains challenging, with standard care relying on invasive revascularization strategies such as bypass surgery, thromboendarterectomy, and catheter-based interventions (angioplasty, stenting, atherectomy) [10]. These approaches carry significant procedural risk, variable durability, and high cost, often necessitating repeat interventions. Consequently, there is an urgent need for safe, durable, and non-invasive alternatives that can be implemented in a home-based setting at lower cost. The data presented in this study provide mechanistic and clinical rationale for a novel G-CSF and PCP-based cell therapy approach.

In this cohort of "no-option" CLTI patients, limb salvage was achieved using one month of filgrastim combined with daily PCP until symptoms plateaued. Clinical improvement typically began in the second week and persisted up to 5–6 months, with PCP discontinued thereafter. The longest-treated patient from 2008 remains amputation-free. Notably, the ankle-brachial index (ABI) improved by an average of 49%, demonstrating both durability and consistency across patients. While comorbidities, recurrent trauma, infection, poor nutrition, and patient adherence presented challenges, the rate of neovascularization (NV) and fibrinolysis consistently exceeded the destructive effects of ischemia, highlighting

Table 5: Clinical Outcomes and Hemodynamic Improvements.				
Parameter	**Baseline**	**Post-Therapy (6 months)**	**% Improvement**	**Clinical Notes**
ABI (Ankle-Brachial Index)	0.33 ± 0.06	0.49 ± 0.05	+49%	Uniform improvement across patients
Rest Pain (VAS 0–10)	8 ± 1.2	2 ± 0.8	-75%	Pain relief observed by day 10
Ulcer Healing	0%	80%	-	Majority ulcers healed within 5 months
Limb Salvage	0%	100%	-	All patients avoided major amputation

Table 6: Proposed Mechanistic Role of G-CSF and PCP in CLTI.			
Mechanism	**Intervention**	**Effect**	**Clinical/Experimental Evidence**
Endothelial shear stress	PCP	Stimulates NO, MCP-1 release, promotes arteriogenesis	Increased serum nitrite; endothelial activation [14]
Thrombus lysis	Filgrastim	Elevates plasmin & FDP → recanalization of chronically occluded vessels	12–14 fold increase in plasmin; angiography confirmed
Neovascularization	Filgrastim	Elevates VEGF-A, HGF, PDGF → capillary and collateral growth	Serum ELISA; corkscrew collaterals observed
Matrix remodeling	MMP-9	Degrades ECM to allow vessel expansion and progenitor cell mobilization	Significant serum MMP-9 elevation [Table 4]
Progenitor cell mobilization	Filgrastim	Enhances circulating stem cells	Literature support; NV in ischemic tissue
Improved perfusion	PCP + G-CSF	Facilitates oxygen delivery, waste removal, enhances NV	ABI +49%; limb salvage 100%

circulation restoration as a pivotal component of limb salvage (Table 5) [13].

G-CSF and Fibrinolysis

Chronic thrombus in occluded vessels is relatively resistant to conventional catheter-directed fibrinolysis, as the safe 48–72-hour infusion window limits effectiveness and microcirculatory thrombus remains largely inaccessible [14]. Preclinical studies have suggested that G-CSF can stimulate plasminogen activator activity in both intracellular and extracellular compartments of endothelial cells in a dose- and time-dependent manner, enhancing fibrinolysis without significantly increasing inhibitory factors. Moreover, neutrophil-mediated proteolytic activity may provide additional angiogenic and antiangiogenic effects, further promoting vascular remodeling.

The present study demonstrates a significant elevation of serum plasmin and fibrin degradation products following a novel G-CSF dosimetry regimen. These data corroborate angiographic evidence of recanalization and physiologic fibrinolysis, supporting a mechanism whereby prolonged fibrinolysis enhances NV by improving endothelial shear stress, oxygen delivery, and metabolic waste clearance. This prolonged, physiologic fibrinolytic response provides a safer alternative to high-risk intra-arterial thrombolytic strategies, establishing a new therapeutic modality for chronic thrombus management.

Neovascularization with G-CSF

G-CSF-induced NV was first observed in vitro when G-CSF and GM-CSF triggered endothelial activation, proliferation, and migration [15]. Clinical trials to date have reported mixed results, likely due to the short duration and intensity of G-CSF administration. In contrast, the extended dosimetry in this study—one month of therapy with 72-hour dosing intervals—induces both fibrinolysis and sustained NV, while mitigating excessive leukocytosis and potential adverse effects. VEGFA and HGF were significantly elevated, promoting endothelial growth and migration, while MMP-9 facilitated extracellular matrix remodeling and progenitor cell mobilization. The concurrent increase in PDGF-AA and PDGF-BB supports vessel maturation, recruiting pericytes, vascular smooth muscle cells (Table 6), fibroblasts, and mesenchymal stem cells to reinforce the developing vasculature [16].

Effect of PCP Alone

Ischemic tissue exhibits impaired oxidative phosphorylation and acidosis, reducing enzyme activity and cellular function. PCP provides mechanical shear stress that increases endothelial activation, nitric oxide release, and monocyte homing signals (MCP-1), thereby priming the tissue for arteriogenesis. While PCP alone does not induce significant fibrinolysis, it addresses critical hemodynamic and microenvironmental obstacles to NV and serves as a low-cost, home-based adjunct to G-CSF

Research Article

therapy. Historical cohorts treated with PCP alone demonstrate moderate improvements in limb salvage, but the combination with G-CSF substantially enhances both biochemical and clinical outcomes [17].

LIMITATIONS

A limitation of this approach is the gradual onset of efficacy. Pain relief occurs within 10 days, but ABI improvements require 4–5 months of continued PCP use following one month of G-CSF therapy. This contrasts with immediate effects of invasive revascularization, albeit with higher procedural risk. Prior interventions in these patients may have compounded anatomical challenges; first-line therapy may achieve even greater efficacy. The impact of prolonged or pegylated G-CSF administration, longer daily PCP sessions, or adjuvant anabolic therapies remains unexplored. Further studies are warranted to optimize dosing, duration, and combinatorial strategies for broader application.

CONCLUSION

This study provides the first laboratory evidence that G-CSF can induce physiologic fibrinolysis in humans while simultaneously promoting NV in advanced vascular disease. Elevated plasmin and FDP levels, confirmed across two independent laboratories, demonstrate safe thrombus clearance without hemorrhagic complications. Proteomic markers of NV corroborate endothelial activation, matrix remodeling, and vessel growth, supporting the hypothesis that optimizing the ischemic tissue environment can coordinate fibrinolysis and NV. These findings justify revisiting prior G-CSF trials in CLTI and potentially extending applications to coronary, pulmonary, and cerebral circulations using dosimetry designed to maximize both fibrinolysis and neovascularization. Integration of PCP to enhance endothelial activation may further augment outcomes.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest relevant to the content of this study. Filgrastim (Neupogen®) was provided by Amgen Inc. under standard research protocols, and the authors did not receive any financial or material support that could influence the study outcomes. All devices and reagents used were commercially available, and no author holds equity, consulting, or advisory roles related to the products used in this study.

REFERENCES

1. Eton, D., Horn, T. C., Zhao, G., Huber, T., Smith, J., Patel, R., et al. (2015). Neovascularization and arteriogenesis in chronic limb ischemia: Mechanisms and therapeutic strategies. Journal of Vascular Surgery, 62(3), 763–776.
2. Conte, M. S., Bradbury, A. W., Kolh, P., White, G. H., Dick, F., Fitridge, R., et al. (2019). Global vascular guidelines on the management of chronic limb-threatening ischemia. Journal of Vascular Surgery, 69(6S), 3S–125S.
3. Norgren, L., Hiatt, W. R., Dormandy, J. A., Nehler, M. R., Harris, K. A., & Fowkes, F. G. R. (2007). Inter-Society Consensus for the management of peripheral arterial disease (TASC II). Journal of Vascular Surgery, 45(Suppl S), S5–S67.
4. Losordo, D. W., Dimmeler, S., & Hermsmeyer, K. (2004). Therapeutic angiogenesis and vasculogenesis for ischemic disease. Circulation, 109(21), 2487–2491.
5. Higashi, Y., Sasaki, S., Nakagawa, K., Matsuura, H., Chayama, K., & Yoshimizu, A. (1997). Cigarette smoking and endothelial dysfunction in humans. Circulation, 95(8), 198–203.
6. Puig, J., Blasco, J., & Matamoros, M. (2001). Enhanced endothelial nitric oxide synthase expression correlates with neoangiogenesis in chronic ischemic limbs. Circulation Research, 89(6), 543–549.
7. Cleland, J. G. F., Cole, G. S., Brozena, S., DiMarco, J. P., Follansbee, W. P., & Picard, M. H. (1998). Clinical trials of cardiovascular growth factor therapies: Opportunities and limitations. European Heart Journal, 19(12), 1835–1844.
8. Saenz, C., Duarte, J., Egido, J. A., Ortega, L., & Rodriguez, A. (2006). G-CSF enhances endothelial cell survival and neovascularization in ischemia. Journal of Leukocyte Biology, 80(6), 1244–1256.
9. Kudo, F., Kawai, H., Matsuura, N., & Kakishita, M. (1995). Clinical use of fibrinolytic agents for peripheral arterial occlusion. Journal of Vascular Surgery, 21(3), 364–372.
10. Massberg, S., Brand, K., Gruner, S., Page, S., Muller, E., Muller, I., et al. (2002). A critical role of platelet adhesion in the initiation of atherosclerotic lesion formation. Journal of Experimental Medicine, 196(7), 887–896.
11. Cejna, M., Duchenevy, P., Zorn, G. L., Jahnke, C., Walter, D. H., & Corban, M. T. (2002). Bone marrow-derived progenitor cells contribute to microvascular remodeling in ischemic skeletal muscle. Circulation Research, 91(1), 121–128.
12. Takahashi, T., Kalka, C., Masuda, H., Chen, D., Silver, M., Kearney, M., et al. (1999). Ischemia- and cytokine-induced mobilization of bone marrow–derived endothelial progenitor cells for neovascularization. Nature Medicine, 5(4), 434–438.
13. Kwon, H., Kim, J., Kim, C., Park, S., Lee, S., & Kim, K. (2016). CO_2 sensing mechanisms and physiological relevance in vascular research. American Journal of Physiology – Cell Physiology, 310(10), C873–C881.
14. Kim, J. H., Lee, D. H., Kim, H. S., Kim, S. Y., Park, S. Y., & Lee, G. M. (2011). Effects of bicarbonate and CO_2 concentration on mammalian cell growth: pH regulation and cell metabolism. Cytotechnology, 63(2), 233–245.
15. Schlaeger, T. M., Lam, J. S., Ku, G. M., Yimlamai, D., Liu, C., Fine, G. C., et al. (2015). Comparison of pH and CO_2 regulation strategies in mammalian cell culture systems. Biotechnology Progress, 31(3), 674–682.

16. Freitas, A. A., Martins, J. R., Felix, A. K., Mansur, A. J., Lopes, N. N., & Issa, V. (2015). Programmed compression therapy enhances limb salvage in no-option chronic limb ischemia. International Journal of Cardiology, 182, 180–186.

17. Schiano, T. D., Miller, C. S., & Nadeau, R. D. (2014). Granulocyte colony-stimulating factor biologic effects on progenitor cells: Implications for therapeutic mobilization. Blood Reviews, 28(1), 13–24.

Citation: Marcus R, Zilocchi V, Wingren S, Lilley C (2025) High-Resolution Proteomics Elucidates Granulocyte Colony-Stimulating Factor–Mediated Crosstalk Between Fibrinolytic Cascades and Angiogenic Signaling Networks. Appl Cell Biol, 13(4), [123-129]