**Advances in Clinical Medicine** 临床医学进展, 2025, 15(4), 958-966
Published Online April 2025 in Hans. https://www.hanspub.org/journal/acm
https://doi.org/10.12677/acm.2025.1541018

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# 中性粒细胞胞外诱捕网在肺动脉高压中的研究进展

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收稿日期：2025年3月3日；录用日期：2025年3月26日；发布日期：2025年4月8日

## 摘　要

**肺动脉高压是一种以肺血管阻力和肺动脉压力升高为特征的临床和病理生理综合征，预后较差，全球疾病负担重。中性粒细胞胞外诱捕网，是一种由活化的中性粒细胞释放至胞外的网状结构，在慢性炎症、血管重塑及血栓形成中发挥重要作用，或可为肺动脉高压的早期识别及治疗提供新思路。因而，本文就中性粒细胞胞外诱捕网在肺动脉高压发生发展中的作用及潜在治疗方案进行阐述。**

## 关键词

**肺动脉高压，中性粒细胞，中性粒细胞外诱捕网**

# Research Progress on the Role of Neutrophil Extracellular Traps in Pulmonary Hypertension

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Received: Mar. 3rd, 2025; accepted: Mar. 26th, 2025; published: Apr. 8th, 2025

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文章引用: 张茜, 罗征秀. 中性粒细胞胞外诱捕网在肺动脉高压中的研究进展[J]. 临床医学进展, 2025, 15(4): 958-966. DOI: 10.12677/acm.2025.1541018

## Abstract

**Pulmonary hypertension (PH) is a clinical and pathophysiological syndrome characterized by increased pulmonary vascular resistance and elevated pulmonary arterial pressure, with poor prognosis and severe global disease burden. Neutrophil extracellular traps (NETs), a network released by activated neutrophils, play a critical role in chronic inflammation, vascular remodeling, and thrombosis, providing novel insights for early identification and therapeutic strategies in PH. Hence, this article reviews the role of NETs in the pathogenesis and progression of PH, as well as explores potential treatment approaches targeting NETs.**

## Keywords

**Pulmonary Hypertension, Neutrophil, Neutrophil Extracellular Traps**



## 1. 引言

肺动脉高压(pulmonary hypertension, PH)是指由多种病因和发病机制导致肺血管结构或功能改变，引起肺血管阻力和肺动脉压力升高的临床和病理生理综合征，可发展成右心衰竭甚至死亡[1]。近期流行病学研究表明，全球 PH 患病率约 1%，其预后较差，造成较重的疾病负担[2] [3]。PH 包含以下 5 个分类：动脉性 PH (pulmonary arterial hypertension, PAH)；左心疾病所致 PH；肺部疾病和(或)低氧所致 PH；慢性血栓栓塞性 PH (chronic thromboembolic pulmonary hypertension, CTEPH)和(或)其他肺动脉栓塞性疾病所致 PH；原因不明和(或)多因素所致 PH [1]。最新国际指南将 PH 定义为：海平面、静息状态下，经右心导管检查测定的平均肺动脉压(mean pulmonary artery pressure, mPAP) > 20 mmHg [4]。右心导管检查作为其诊断金标准，由于其有创性，在早期诊断中具有局限性；而超声心动图及部分生物标志物在诊断准确性及特异性上存在不足。现有 PH 靶向治疗仍存在疗效欠佳、不良反应严重等局限性。因而，寻求 PH 无创早期诊断工具及新的治疗靶点具有重要临床意义。

中性粒细胞胞外诱捕网(neutrophil extracellular traps, NETs)是由活化的中性粒细胞释放至胞外的一种特殊大分子复合体，其网状结构包括骨架成分双链 DNA (double-stranded DNA, dsDNA)及组蛋白、髓过氧化物酶(myeloperoxidase, MPO)、弹性蛋白酶(neutrophil elastase, NE)等多种颗粒蛋白。在 NETs 形成过程中伴随着中性粒细胞的死亡，被称为 NETosis [5]。近年国内外研究发现肺动脉高压患者循环 NETs 及其标志物水平升高，NETs 在肺动脉高压的发生发展中具有一定作用[6]。本综述针对 NETs 在肺动脉高压的发生发展中的作用进行阐述，或可为 PH 的早期识别及治疗靶点提供新思路。

## 2. NETs 与 PH 的关系

2016 年，Albabbous 团队首次在 iPAH 患者的肺组织中发现 NETs [7]：DNA、MPO 及瓜氨酸组蛋白酶 3 (citrullinated histone H3, CitH3)在闭塞性丛状病变中富集；且 NETs 形成的关键酶肽基精氨酸脱亚胺酶 4 (Peptidylarginine Deiminase 4, PAD4)阳性的中性粒细胞分布于重塑血管壁及血管周围炎性浸润区。此后，国内外研究进一步证实了 PAH 患者及动物模型中 NETs 及其标志物水平升高，并探究了 NETs 在各

类 PH 中的作用。既往研究发现，PAH 患者血浆 NE 与疾病严重程度及不良预后相关[8]，血浆及肺组织中 MPO 水平与血液动力学恶化、心功能下降及生存率降低等不良预后事件相关[9]。而在动物模型中，抑制 NE 可逆转血管及心脏重构，促进内皮功能正常化及心功能恢复，降低死亡率[10]-[14]。近期研究发现，PAH 患者血浆 NE 和内源性逆转录病毒包膜蛋白包膜(HERV-K)水平增高，并可诱导小鼠肺动脉高压，而 NE 抑制剂及抗病毒药物 elafin 可缓解这一进展，提示 NETs 与肺动脉高压的发生发展有关[15]。外周血中性粒细胞与淋巴细胞比值(neutrophil to lymphocyte ratio, NLR)升高是 PAH 和 CTEPH 的独立不良预后指标，提示中性粒细胞过度活化与疾病恶化相关[16]-[19]。有研究探讨了 NETs 在动静脉系统中的作用，提示了其在 CTEPH 发病机制中的作用[20]。Kevin Didier 等发现，系统性硬化相关肺动脉高压患者中 NETosis 持续存在[21]。在先天性心脏病相关肺动脉高压患者中，抑制 NE 活性可显著减轻肺损伤[22]。近期研究发现了由 TLR4 /NLRP3 信号通路介导的内皮细胞铁死亡可诱导大鼠 PH [23]，而 NETs 与铁死亡之间存在的联系[24] [25]进一步提示了 NETs 与 PH 的关系。

## 3. NETs 在 PH 中的病理机制

慢性炎症和血管重塑是 PH 的病理表现和发病因素之一[4]。既往研究提示，NETs 不仅在慢性炎症中具有重要作用，也可导致内皮功能障碍、促进血管平滑肌细胞(vascular smooth muscle cells, VSMCs)的迁移和增殖、血管生成及血栓形成[6] [26]，可能引起肺动脉管腔进行性狭窄、闭塞，肺血管阻力升高，从而引发肺动脉高压。

### 3.1. NETs 促进血管炎症和内皮损伤

研究发现 PH 患者中性粒细胞中 Rho 激酶通路异常激活[27]，Rho 相关的卷曲螺旋激酶(Rho associated coiled coil-forming kinase, ROCK)可显著上调包括白细胞介素(IL)-6、趋化因子配体 2 (C-C motif chemoattractant ligand 2, CCL2)和肿瘤坏死因子(TNF)-$\alpha$ 等促炎细胞因子的表达，放大炎症级联反应[28,29]，驱动肺血管微环境的慢性炎症；而 TNF-$\alpha$、IL-1、IL-6 等促炎因子可导致内皮型一氧化氮合酶活性降低，内皮一氧化氮释放减少，使得一氧化氮依赖性松弛反应减弱，导致内皮舒张功能障碍，另一方面可进一步促进中性粒细胞募集，形成炎症及内皮损伤的恶性循环。既往文献报道，MPO 主要通过以下两个途径导致内皮功能障碍：激活 Rho 激酶信号通路，导致内皮功能障碍[9] [27]；与 NADPH 氧化酶产生的活性氧(reactive oxygen species, ROS)协同作用，形成肺血管床强氧化微环境[30]，导致内皮型一氧化氮合酶失调，直接损伤内皮细胞(endothelial cells, ECs) [31]，引起血管舒张障碍和重塑增强。此外，氧化应激在实验条件下可诱导 NETs 释放[32]，形成正反馈。NE 可通过降解弹性蛋白，释放弹性蛋白衍生肽(Elastin-derived peptides, EDP)，其中具有促炎活性的组分可直接刺激炎症反应；另一方面激活内皮细胞释放 IL-8、CCL2 等趋化因子，募集更多中性粒细胞，形成“NE-炎症”正反馈循环[33]；还可诱导基质金属蛋白酶(matrix metalloproteinases, MMPs)分泌[34]，破坏肺血管基质，释放炎性介质，从而导致内皮损伤。NE 还可通过抑制 BMPR2 信号通路，导致肺动脉 ECs 过度凋亡[8]。此外，NE 可与 MPO 协同作用产生 ROS [35]，加重内皮功能障碍。

### 3.2. NETs 参与肺血管重塑

NE 通过抑制 BMPR2 信号通路，导致 VSMCs 过度增殖[36]。NE 还可刺激 MMPs 分泌[34]，通过降解胶原蛋白、层粘连蛋白等细胞外基质(extracellular matrix, ECM)成分，增强细胞迁移能力，促进血管重塑及新生血管形成[37] [38]。此外，弹性蛋白降解产生的 EDP 可直接激活 ECs [33]和 VSMCs 增殖[33] [39]，诱导肺血管壁增厚及新生血管形成。研究表明，NE 活性降低与血管重构逆转相关[10]-[15]，进一

步论证了 NE 在肺血管重塑中的作用。MPO 和 $H_2O_2$ 激活内皮细胞的 Toll 样受体(TLR) 4 导致 NF-$\kappa$B 表达上调，细胞间黏附分子(ICAM)-1、MMP9、肝素结合 EGF 样生长因子(HB-EGF)等促内皮迁移及血管生成因子释放，诱导新生血管形成，驱动血管重塑[7]。MPO 还可通过与 ROS 协同作用，形成肺血管床强氧化微环境[30]，通过 SMAD、MAPK、Rho-GTPase 等信号通路活化调节转化生长因子 $\beta$ (transforming growth factor $\beta$, TGF-$\beta$)信号通路，发挥促纤维化作用，促进血管重塑[31]。此外，MPO 抑制剂在动物模型中可减轻氧化损伤和血管重构[40]，也进一步论证了 MPO 在肺血管重构中的作用。

中性粒细胞中 Rho 激酶通路的异常激活[27]，可显著上调 IL6 等促炎细胞因子的表达[28]；此外，研究发现 PAH 患者中 IL-1$\beta$、IL-8 水平显著升高[29]。而 IL-1$\beta$、IL-6 具有诱导 VSMCs 增殖、减少其凋亡的作用，可导致肺血管壁增厚和管腔狭窄[41]-[43]；IL-8 可增强 ECs 存活能力、增加 MMPs 的表达[44]，进而促进血管生成，构成 PAH 的“炎症－血管重塑”恶性循环。此外，NETs 可通过增强 ECs 内皮素(ET)-1 的表达，刺激 VSMCs 异常增殖，促进血管壁增厚，加速血管闭塞性病变[7]。最近的研究表明，NETs 可以通过激活由卷曲螺旋结构域 25 (CCDC25)介导的 ILK/$\beta$-parvin/RAC1 通路刺激肺动脉 VSMCs 的细胞骨架重塑和表型转化[45]，从而影响该细胞功能[46]，对 PAH 的进展至关重要[47] [48]。

### 3.3. NETs 与血栓形成

肺动脉高压患者普遍存在血栓性病变及凝血功能异常[49]。研究显示 MPO 和 NE 可直接激活凝血因子，并抑制抗凝通路[50] [51]。NETs 来源的组蛋白及 MPO 可激活内皮细胞，上调组织因子和黏附分子表达，进一步放大内皮活化、促进血栓形成[52]。NETs 来源的组蛋白及 MPO 可刺激血小板活化和脱颗粒，活化的血小板通过 P-选择素/PSGL 介导中性粒细胞活化，并通过整合素-GP1b 和 CCR1-CXCL4/CCR5-CCL5 相互作用或 HMBG1 信号传导诱导 NETosis，不断释放 NETs[26]。NETs 作为网状结构，可与血小板结合并促其聚集，为血栓提供物理支架[53]，增强血栓稳定性[54]。活化的中性粒细胞与血小板两者的相互作用形成正反馈，最终导致血栓形成、血管重塑和血管收缩。前文提到，NE 可与 MPO 协同作用产生 ROS [35]，而研究表明 ROS 可促进血栓形成[55]。此外，Kesturu S. Girish 团队发现，铁死亡介导的 NETosis 加速了 CTEPH 患者中的肺栓塞形成。不仅如此，NETs 还通过 TGF-$\beta$ 信号通路诱导血栓纤维化，从而导致血栓负荷增加和消除延迟，由静脉血栓转化为纤维化血管阻塞，从而导致 CTEPH [56]。研究发现 NETs 降解酶脱氧核糖核酸酶(DNase) I 可促进血栓溶解[51]，也进一步提示了 NETs 与血栓形成的关系。

## 4. 靶向 NETs 的潜在治疗策略

最近的研究强调了 NETs 在 PH 发病机制中的重要作用。尽管目前针对 NETs 治疗 PH 的研究较少，现有研究已经在细胞实验和动物模型中提示了其治疗作用，抑制 NETs 可以改善病情、逆转疾病进展。因而，NETs 有望成为未来 PH 治疗的潜在靶点，但针对该靶点的 PH 治疗安全性及有效性尚不明确，其是否能推广至临床实践中还需要更多高质量基础研究及临床研究验证。

### 4.1. 直接降解 NETs

DNA 是 NETs 的骨架成分，因而作为其裂解的关键酶，DNase I 是降解 NETs 过程的重要因素。在既往研究中，DNase I 常被用于抑制 NETs [57] [58]。Farrera 等的研究表明，NETs 的降解依赖于 DNase I 和巨噬细胞。高浓度 DNase I 可有效降解 NETs，而生理浓度 DNase I 无法催化这一过程，可能通过将 DNA 主链分解成更易处理的片段，从而增强巨噬细胞对 NETs 的清除[59]。此外，研究发现 DNase I 可促进血栓溶解，进而延缓 PH 进展[51]。

### 4.2. 抑制 NETs 形成

PAD4 是形成 CitH3 的关键酶。该酶介导组蛋白上精氨酸残基转化为瓜氨酸，移除核心组蛋白的正电荷，从而削弱组蛋白与 DNA 之间的静电相互作用，导致解聚的染色质 DNA 释放，参与 NETosis [60]。PAD4 抑制剂由于其独特的化学结构，表现出对 PAD4 的强特异性[60]。现有研究提示了该抑制剂在心血管疾病中的潜在治疗作用[61]-[63]，但目前尚无探讨 PAD4 抑制剂对 PH 疾病进展影响的相关研究。此外，如前文所述，NE 抑制剂可减低肺动脉压力，逆转血管重构[11] [15]；MPO 抑制剂可减轻氧化损伤和血管重构[9] [64]。目前，一项注册号为 NCT03522935 临床试验已进入实施阶段，旨在系统评估 elafin 在抗 PH 治疗中的安全性与有效性，该药物有望被推广至 PH 的临床治疗。前列环素由血管内皮细胞产生，具有强效扩张血管的作用，是 PH 的特异性治疗，有研究提出了其通过直接抑制中性粒细胞的脱颗粒过程及 NE 释放改善 PAH 病情的额外机制[65]。

ROS 在 NETs 生成中起着重要作用[66]。Zeng 等发现，抗氧化剂能够有效抑制激活的中性粒细胞产生 ROS，并阻碍依赖 ROS 的 NETs 形成[67] [68]。肝素是一种具有抗凝活性和抗血栓作用的多功能糖胺聚糖，可抑制中性粒细胞的活化，从而减少 NETs 的产生[69] [70]。此外，研究提示他汀类药物和二甲双胍可以有效抑制 NETs 的形成，降低血浆中 MPO 等 NETs 标志物水平，并减少外周血中性粒细胞的数量，还可通过抑制 NETosis 相关的炎症改善心力衰竭[71] [72]。

### 4.3. 联合治疗策略

多项临床研究证明 PH 靶向药物序贯联合治疗较单药治疗能取得更好疗效[73]-[75]。因而，NETs 靶向药物与内皮素受体拮抗剂、磷酸二酯酶 5 型抑制剂、前列环素等传统 PAH 靶向疗法结合，或许是 PH 治疗的新方向。

## 5. 小结

综上所述，肺血管周围炎症反应及血管重塑是 PH 的关键致病因素。NETs 可通过不同机制引起慢性炎症、内皮功能障碍、血管重塑及血栓形成，在 PH 发生发展中具有重要作用。但目前研究主要集中于 PAH，对其他类型 PH 的相关研究尚存在不足，因而，NETs 在 PH 不同亚型中的特异性作用仍需进一步探索。且 NETs 的检测存在异质性，在 PH 诊断的临床应用上仍存在较大距离。治疗方面，其作为新型治疗策略虽具有一定潜力，也面临免疫抑制等风险，具有一定挑战性。未来仍需开展多类型临床及基础研究，进一步探索其作用机制及临床应用价值，推动基础与临床研究的转化。

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