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COMMENTARY

Leveraging vascular normalization *via* eNOS activation to enhance the efficacy of PD-L1 blockade

KEY WORDS

eNOS activation;
Vascular normalization;
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Featured with irregular, leaky, and distorted structures, abnormal tumor vasculature not only meets the metabolic demands of tumor growth and metastasis, such as the supply of oxygen and nutrients, but also fosters an immunosuppressive microenvironment[1]. This compromised vascular state undermines the effectiveness of immunotherapies such as programmed cell death ligand 1 (PD-L1) blockade. The past twenty years have witnessed the shift from anti-angiogenic therapy to vascular normalization, a more refined approach that improves drug delivery and helps reverse immunosuppression. Within this context, nitric oxide (NO) serves as a key gaseous signaling molecule and vascular modulator for tumor therapy[2,3]. Although delivering NO donors could partially restore perivascular NO gradients, the insufficient accumulation in tumor endothelial cells would compromise the efficacy of vascular normalization, and off-target distribution would even exacerbate that, which remain the dominant challenges for the clinical translation of tumor vascular normalization.

Instead of directly delivering NO donors, a more promising strategy for sustained vascular normalization is to enhancing the endogenous enzymatic function of endothelial nitric oxide synthase (eNOS) in tumor vasculature. In a recent study published in *Acta Pharm Sin B*, Wu et al. developed a simple lipid nanoparticle (MC@L) co-delivering CaO_2 and metformin (Met), achieving coordinated multi-target effects[4]. This "kill-two-birds-with-one-stone" design acted on two cell types: in vascular endothelial cells, released Ca^{2+} and Met synergistically activated eNOS *via* coupling and phosphorylation, restoring physiological perivascular NO gradients to normalize vessels. In tumor cells, CaO_2 decomposition induced immunogenic cell death (ICD) while alleviating hypoxia and acidosis. Concurrently, Met downregulated the tryptophan (Trp) transporter SLC7A5, which decreased the transformation of Trp into immunosuppressive kynurenine (Kyn), to relieve immunosuppression. This multifaceted approach demonstrated superior antitumor efficacy when combined with PD-L1 blockade therapy. That was a smart design through the simplest lipid nanoparticles, making the best of CaO_2 and Met in two types of cells, vascular endothelial cells and tumor cells for vessel normalization and immunosuppression reversion.

Vascular normalization by MC@L was largely attributed to the pH-responsive release of Ca^{2+} and Met. The on-demand release profile is the prerequisite for their function. The degradation of MC@L was accelerated under acidic conditions, accompanied by a dose-dependent increase in solution pH and oxygen generation, confirming its ability to ameliorate tumor acidosis and hypoxia. Central to this design was the activation of eNOS and endothelial transcytosis. Western blotting results indicated MC@L significantly enhanced eNOS phosphorylation at Ser1177 and increased NO production in HUVECs. Compared with Met or CaO_2 alone, MC@L reduced tube length and branches more effectively, while upregulating the maturation marker angiopoietin-1 (Ang 1). Intracellular and extracellular content of Cy5 decreased and increased, respectively, with the time incubating, verifying the successful transcytosis of MC@L, consistent with the TEM results. Notably, MC@L group exhibited the highest lectin perfusion, attributable to enhanced NO generation and vascular maturation. As seen in the previous studies, low or high doses of NO donors was found to influence tumor angiogenesis in a different way[5,6]. Low-dose NanoNO treatment can increase promote vascular normalization, pericyte coverage, and improved

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perfusion, whereas high doses exert anti-angiogenic effects. In addition, the off-target accumulation of NO donors might trigger harmful side-effects. In contrast, the present strategy focused on localized, endogenous NO generation *via* eNOS activation within tumor vascular endothelial cells, achieving precise spatial control. As expected, eNOS activation-mediated vascular normalization increased the infiltration of activated immune cells, and combined with CaO_2-induced ICD and Met-mediated inhibition of Trp uptake in tumor cells potently reversed the immunosuppression, thus remarkably improving PD-L1 blockade efficacy.

Despite its promise, this Ca^{2+}/Met-mediated eNOS activation strategy *via* lipid nanoparticles shares two persistent challenges with conventional NO donor delivery, which are critical for vascular normalization and clinical translation. First, its therapeutic outcome—whether anti-angiogenic or normalizing—is highly dose-dependent. The off-target accumulation might result in unintended NO production in healthy vasculature of normal tissues, leading to potential toxicity. In future research, it needs to explore a reliable and safe dosage window of nanoparticles to achieve consistent vascular normalization. Second, enhancing the targeting specificity toward tumor neovessels while minimizing distribution in normal tissues remains essential[7]. Beyond spatial targeting, eNOS activation should ideally be confined to or preferentially occur in neovessels rather than in normal vessels within the tumor microenvironment. If these issues are likely to be successfully addressed, this nanoparticle-enabled strategy could offer a novel and transformative therapeutic alternative for tumor vascular normalization. With further development, it holds considerable potential to improve outcomes in solid tumor therapy, particularly in combination with immunotherapy.

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Author contributions

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Conflicts of interest

The authors declare no conflicts of interest.

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