Mini-Review

The anti-metastasis effect of low-dose carbon monoxide

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Abstract: Metastasis is the primary cause of cancer-related deaths. Because of the anatomically diffused localizations in different organs, treating metastatic cancer with conventional surgery or radiotherapy is challenging. Systemic therapies, including chemotherapy, immunotherapy, and targeted therapy, are used for metastatic cancer treatment. However, resistance to chemotherapy often develops over time, and immunotherapy or targeted therapy is not available for every cancer type. Improved systemic therapies for metastatic cancer are urgently needed. Among all forms of cancer, pancreatic ductal adenocarcinoma (PDAC) is a lethal type due to the following characteristics: early distant metastasis, abundant desmoplastic stroma, insufficient therapeutics, and frequent chemoresistance. Carbon monoxide (CO) is produced endogenously in humans and has been established as an important, biologically active signaling molecule. Accumulating evidence suggests the therapeutic applications of safe, low-dose CO for various diseases. Recently, low-dose CO was shown to suppress metastasis of PDAC in an orthotopic mouse model of liver metastasis and a spontaneous PDAC metastasis mouse model after the removal of primary tumor xenograft tumors. In this mini-review, we discuss the findings and the potential development of CO-based systemic therapies for metastatic cancer. Specifically, many non-inhalation CO delivery modalities with easy control of dosages have been developed and can be further studied for their anti-metastatic properties.

Keywords: Metastatic cancer; pancreatic ductal adenocarcinoma; carbon monoxide

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Most cancer deaths are due to metastasis and its negative impacts on the function of vital organs. At the time of primary cancer diagnosis, tumor cells in many patients have already spread to distant organs and will become metastatic disease if these microscopic foci of disseminated tumor cells (microscopic metastases) are left untreated. Once diagnosed with metastatic disease, it is notoriously difficult to treat. Pancreatic cancer is projected to become the second leading cause of cancer-related death by 2030. About 48% of pancreatic cancer patients are diagnosed with distant metastases (1), with the liver being the most common site of metastasis. Pancreatic cancer can be divided into exocrine tumors and pancreatic neuroendocrine tumors (PNET). The majority of pancreatic cancer is pancreatic ductal adenocarcinoma (PDAC), one type of exocrine tumors. PDAC is among the most aggressive of all cancers. Potentially curative surgical resection is limited to those PDAC patients diagnosed at an early stage, yet many of them ultimately experience recurrence (2). For those with metastatic PDAC, standard chemotherapy only marginally extends survival. Overall, the 5-year survival rate of PDAC is ~11% (1), underscoring the urgent need for novel therapies.

Carbon monoxide (CO) is an important cell signaling molecule produced endogenously by heme degradation (3). Research has uncovered therapeutic use for CO when applied at low concentrations for various non-cancerous diseases (4-6). Clinical trials of inhaled CO to treat pulmonary diseases demonstrated no adverse events after 12 weeks of inhaled low-dose CO therapy (7). Low-dose CO has therapeutic potential in inhibiting primary tumor growth in mouse models and sensitizing chemotherapeutic

agents (8). Vítek *et al.* (9) reported that 500 ppm CO (1 hour daily) reduced the subcutaneous tumor burden of human PDAC cell line xenograft in athymic mice and increased the survival rate twofold compared with the control mice. However, the subcutaneous tumor mouse model cannot address the metastatic nature, important determinant of the therapeutic response.

In a recent study, Zhang *et al.* (10) provided the direct evidence for the effect of low-dose CO (250 ppm, 3 hours daily) in suppressing metastasis using two clinically relevant mouse models. First, the authors demonstrated that low-dose CO significantly inhibited liver metastasis of PDAC using an orthotopic mouse model of liver metastasis by intrasplenically injecting human PDAC cell lines (11). Treatment of 250 ppm CO (3 hours daily) significantly blocked the metastatic outgrowth of PDAC in the liver. Second, because of the high recurrence rates in early-stage PDAC patients despite surgical resection followed by chemotherapy (12), the authors investigated the use of low-dose CO as an adjuvant therapy to prevent the progression of microscopic metastasis to gross metastasis. To mimic the clinic setting, the authors developed a new spontaneous PDAC metastasis mouse model by growing human PDAC cells subcutaneously in NOD/scid-lL2Rgc knockout (NSG) immunodeficient mice and then resecting tumors when their sizes reached 0.4 cm in diameter. In this model, mice succumbed to recurrence with PDAC metastases in the liver and the peritoneal cavity 4 weeks post-surgery. Importantly, the authors found that low-dose CO treatment significantly suppressed recurrence after surgical removal of primary PDAC. In addition, the authors demonstrated the therapeutic effect of low-dose CO in blocking lung metastasis in a triple-negative breast cancer mouse model, which broadens the impact of low-dose CO therapy in treating other aggressive metastatic cancer types. Of an important note, mice treated with low-dose CO (250 ppm, 3 hours daily) in all three metastasis mouse models examined by Zhang *et al.* remained healthy when the untreated mice became lethargic and needed to be euthanized as metastatic diseases developed.

Mechanistically, Zhang *et al.* found that low-dose CO blocked the transcription of heme importers, *HRG1* and *HCP1*, leading to diminished intracellular heme levels, a heme-regulated enzyme, cytochrome P4501B1 (*CYP1B1*), and its downstream molecules, including *SP1*, Myc, and Myc target genes (10). It has been reported that elevated heme levels are found in cancer cells and contribute to tumorigenesis (13,14); heme upregulates c-Myc expression in prostate cancer (14); siRNA against *HRG1* impairs migration of HeLa cells (15); CYP1B1 promotes migration and invasion (16); CYP1B1 overexpression is associated with poor response to chemotherapy (17). These earlier studies indicate the roles of heme, Hrg1, and CYP1B1 in metastasis. Zhang *et al.* showed that either supplementing heme or overexpressing CYP1B1 reverses the anti-migration effect of low-dose CO *in vitro*. Because Myc regulates cancer metabolism (18), the authors performed polar metabolite profiling by liquid chromatography-mass spectrometry (LC-MS) and a pathway topology analysis through which they found that the TCA cycle was significantly downregulated in the CO-treated cells. On the contrary, hypoxia was not induced in the low-dose CO-treated tumor cells as measured by HIF1α and HIF2α protein levels. Whether low-dose CO therapy achieves its anti-metastatic effect by downregulating intracellular heme levels, the CYP1B1/Sp1/Myc axis, or the TCA cycle *in vivo* and in various cancer types requires further investigation (*Figure 1*). Heme is a regulatory molecule exerting functional consequences via transient binding to many proteins. Besides CYP1B1, other heme-regulated proteins may contribute to the anti-metastatic effect of low-dose CO. Taken together, this work by Zhang *et al.* provides strong evidence to repurpose low-dose CO for the inhibition of metastatic cancer development.

Looking into the future, we see great potential for developing CO as a therapeutic agent against metastasis. On the one hand, the property of CO makes it an excellent candidate for systemic therapy of metastatic cancer. CO is very stable and is known to be highly permeable and diffusible through the endothelium [review in (3)]. Additional investigation is needed to determine whether low-dose CO can lead to the regression of metastatic cancer. As the immune system is increasingly recognized to play a critical role in cancer progression and therapeutic efficacy, this novel therapeutic concept needs further investigation using immunocompetent animal models. On the other hand, there is a need to develop non-inhalation CO delivery methods to allow for easy and controlled CO administration. Along this line, there have been intensive efforts in developing various CO delivery forms (19-25). Especially interesting to us is a group of organic CO prodrugs with tunable and controllable release properties (21,26,27). These organic CO prodrugs have been extensively studied for their pharmacological effects in models of kidney injury (26), liver injury (21,28), chemically induced gastritis (29), colitis (21), and general inflammation (28). *Figure 2* shows some examples. Furthermore, the pharmacokinetic behaviors of

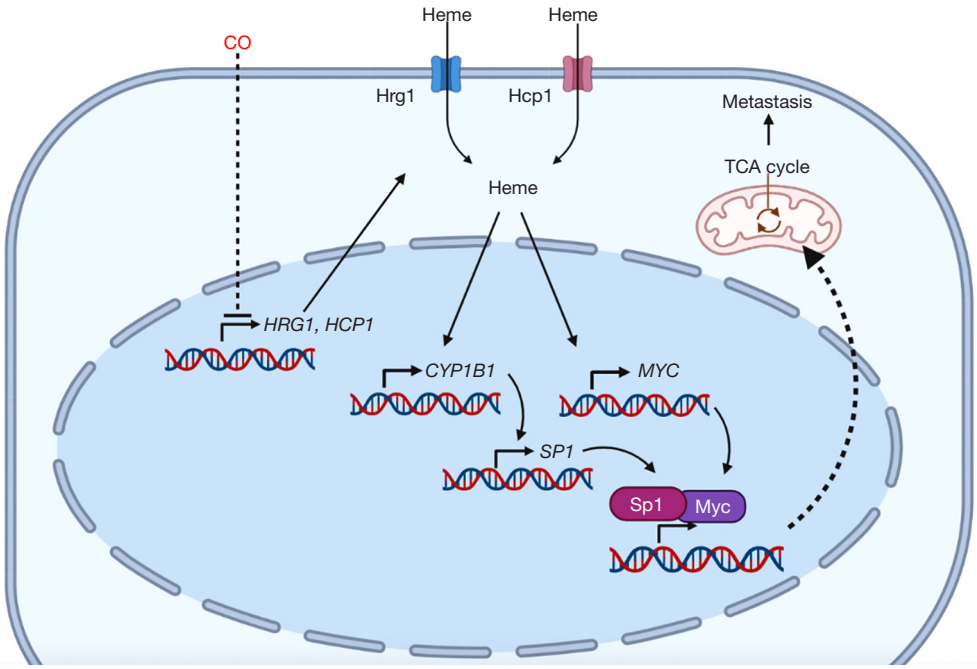
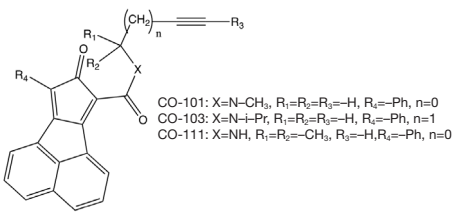
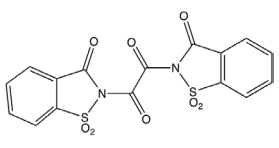


Figure 1 A working model by which low-dose CO suppresses metastasis (created in BioRender.com). CO, carbon monoxide.





CO-306, a prodrug with saccharine as the "CO carrier"

Prodrug	Animal model studies	CO-release half-life
BW-CO-101	Kidney ischemia reperfusion injury	2 min (21)
BW-CO-103	Colitis	1.2 h (21)
BW-CO-111	Gastric protection	0.2 h (21,29)
BW-CO-306	Acute kidney injury	1.3–21 min depending on the formulation (26,27)

Figure 2 Selected CO prodrugs and their basic properties. CO, carbon monoxide.

some of these prodrugs have been extensively characterized (27,30). The application of these alternative delivery forms in studying CO's effects on metastatic cancer prevention and treatment will hopefully lead to candidates for the clinical development of CO-based systemic cancer therapies.

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Footnote

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at https://apc.amegroups.com/article/view/10.21037/apc-2022-4/coif). YCND is The Rasweiler Family Research Scholar in Cancer Research and received the following grants: NIH R01CA204916-

01A1, DoD W81XWH-16-1-0619, and STARR I12-0043. YCND's team has Utility Application filed on CO and metastasis. CO prodrug-related work in the lab of BW has been supported by the National Institutes of Health (DK119202 on CO and colitis; and DK128823 on CO and acute kidney injury), the Georgia Research Alliance Eminent Scholar endowment fund (BW), and internal resources at Georgia State University. BW's team has several patents filed or approved on the CO prodrug work. The authors have no other conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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