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INVITED COMMENTARY

CO_2 to Live and to Die

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ABBREVIATIONS

CA = carbonic anhydrase, ECM = extracellular matrix, HIF = hypoxia-inducible factor, IO = interventional oncology

In their study, Ueshima et al (1) have demonstrated that infusion of CO_2-saturated solution, at least in the short term (3 days), resulted in growth inhibition in a rabbit thigh VX2 tumor model by activation of apoptotic cell death through cleaved caspase-3 upregulation (1). The following three mechanisms are suggested to explain their results: (i) mitochondrial DNA apoptosis induced via intrinsic pathways by increasing intracellular $Ca2^+$ and reactive oxygen species, (ii) an increase in intracellular acidity, and (iii) a stronger Bohr effect (ie, increase in

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partial pressure of CO_2 or lower pH will cause offloading of oxygen from hemoglobin, which allows transport of oxygen to tissues), resulting in improvement of cellular hypoxia in the tumor (1).

Based on the results of this study (1), perhaps it is early to forecast CO_2-saturated solution as a viable embolic agent in treating or sensitizing tumor cells to locoregional or systemic treatment. However, the concept is indeed intriguing and is one that could lead to a better understanding of cancer development and progression and new opportunities for innovation in interventional oncology (IO).

CO_2 is a colorless, odorless gas that is soluble in water, ethanol, and acetone, with a melting point of $-55.6°C$ and a boiling point of $-78.5°C$. Also, CO_2 is an essential organic compound that provides the basis for the synthesis of other organic compounds that provide nutrients for plants and animals. Although physical properties such as solubility of CO_2 allows it to be used as a safe negative contrast agent, its roles in tumor physiology and microenvironment are less known and yet to be exploited as a tool in IO (2).

CO_2 and hypoxia play an important role in unique microenvironment of many solid tumors and represents a major source of therapeutic failure in cancer therapy (3). The importance of hypoxia in tumor biology could be tracked back to an observation by Warburg et al (4) —ie, the Warburg effect—that demonstrates that, unlike normal cells, tumor cells favor glycolysis, independent of cellular oxygenation levels. Enhanced anaerobic glycolysis capacity allows tumor cells to survive and grow in hostile microenvironment with poor vascularization and decreased perfusion, adapting to oxygen and nutrient deprivation (5,6).

It is believed that fluctuating blood flow in tumors very frequently gives rise to alternating regions of hypoxia; therefore, tumor microenvironment often changes, which may play a role in cancer stem cell plasticity (7). Also, it was recently postulated that there is a symbiotic relationship between hypoxic tumor cells and oxygenated tumor cells and stromal cells in the use of lactate as a preferential oxidative fuel, ie, "lactate shuttle" (8,9). CO_2 not only plays a significant role in tumor microenvironment, but also likely plays a key role in cancer development. Under intermittent hypoxia conditions, reactive oxygen species are generated, and overexpression of hypoxia-inducible factor (HIF)–1α leads to an up-regulation of proangiogenic mediators (8). These mediators are involved in carcinogenesis, increasing tumor vasculature, and accelerating tumor growth (8,10,11). Supporting this concept, increased overnight hypoxia in patients with sleep apnea syndrome has been associated with increased cancer incidence in men younger than 65 years of age (12).

Apart from direct impact on tumor angiogenesis and metastasis through HIFs, hypoxia also has been shown to induce epithelial-to-mesenchymal transition (ie, loss of cell–cell contact) and promote cancer stem cells. Cycles of hypoxia and reoxygenation have been shown to enhance proliferation of cancer stem cells (7,13,14). HIF-1 is a transcriptional factor that mediates multiple adaptive responses to tumoral hypoxia, such as reprograming cell metabolism, cell proliferation, angiogenesis, and invasion by regulating the number of downstream protein-coding genes, including vascular endothelial growth factor, cysteine-X-cysteine receptor 4, and microRNAs (12,15,16).

Recently, microRNAs have been implicated in upstream and downstream signaling of HIF pathways. Among hypoxia-regulated microRNAs, miR-210 has been identified as a master regulator of tumor hypoxic response. miR210 has been shown to have several biologic targets affecting cell arrest cycle, stem cell survival, mitochondrial metabolism, DNA repair, angiogenesis, cancer metastasis via vacuole membrane protein 1 (VMP1), and susceptibility of tumor cells to lysis by cytotoxic T cells (15,17,18). Hypoxia also aids in tumor escape from immune surveillance by suppressing maturation of dendritic cells, promoting a negative immunoregulatory cell population with tumor, and recruiting monocytes and rapidly differentiating them into tumor-associated macrophages (10).

Hypoxia also drives a protumor extracellular matrix (ECM) remodeling via increase in expression of matrix remodeling enzymes such as matrix metalloproteinases and proteins such as lysyl oxidase, thereby increasing ECM viscoelasticity. In addition, hypoxia disrupts tissue integrity and enhances cell motility by directly repressing E-cadherin and enhancing N-cadherin (10,19,20).

As a result of the Warburg effect, cancer cells have higher intracellular pH and lower extracellular pH compared with normal adult cells. Acidity in tumor environment promoted by accumulation of H^+ and lactate in extracellular space also is shown to be detrimental in tumor resilience. Tumor environment acidity is accountable for chemotherapy resistance by impairing drug uptake or neutralizing or sequestering weakly basic chemotherapeutic agents (eg, doxorubicin) into intracellular vesicles (7,21,22). Recently, it has been demonstrated that doxorubicin can induce HIF-1α accumulation in normoxic cells, limiting its efficacy (23). Carbonic anhydrase (CA) IX, a pH-regulating hypoxia-induced enzyme, also plays a role in cell spreading and migration. Similarly, CA XII expression has been up-regulated under hypoxic conditions in many human tumors, facilitating CO_2 venting and intracellular pH regulation (24).

Given the integral role of CO_2 in tumor generation, survival, and progression, targeting physical properties of cancer microenvironment and manipulating partial O_2 and CO_2 pressures, acidity, and ECM stiffness could be proven as safe and effective methods in treating and sensitizing tumor cells in targeted therapies (25).

In addition to the report by Ueshima et al (1), there are several preliminary laboratory and animal studies that support the possible effectiveness of these methods (26–29). CO_2 therapy has been shown to significantly reduce expression of HIF-1α and vascular endothelial growth factor in human malignant fibrous histiocytoma, likely via reduced hypoxia in treated tumor tissue, probably as a result of an artificial Bohr effect (26). In addition, changes in CO_2 concentration can increase the invasive ability of colon cancer cells (27). A recent report by Liu et al (28) showed enhancement of cisplatin-based chemoembolization by a hemoglobin-based oxygen carrier, OC89, in an orthotopic rat hepatocellular carcinoma model. OC89 effectively attenuated the hypoxia of hepatocellular carcinoma tissue (de novo and treatment-induced) and enhanced the efficacy of cisplatin-based chemoembolization in their model (28). New-generation treatment based on tumor microenvironment such as hypoxia-activated prodrugs and drugs targeting the tumor hyperglycemic environment are being developed (29). Also to exploit tumor hypoxia for targeted therapy, the YB1 strain of *Salmonella typhimurium* has been engineered to grow under hypoxic condition and be able to produce tumor necrosis factor, HIF-1α antibody, or other oncolytic biomolecules (30). Similarly, monoclonal antibodies against CA have been shown to have antitumor effects (31).

Of interest, cancer resistance in species such as *Spalax* and *Heterocephalus*—both subterranean long-lived rodents—with known tolerance to extreme hypoxia offers further proof of the presence of genes and mechanisms that overlap in cancer resistance, apoptosis, angiogenesis pathways, and hypoxia tolerance. These animal models might be useful in testing new concepts and techniques based on manipulation of physical properties of tumor microenvironment (32).

It is important to remember, although inducing a hostile environment (ie, hypoxia) with conventional systemic and locoregional treatment results in cell death in certain cancer cells, it also triggers adaptation for survival (ie, "stay") and leaving in search of better conditions (ie, "escape") via HIF and other pathways. Therefore, a combination and a tempered therapeutic target (ie, a strategy to contain rather than kill) might be more helpful in overcoming evolutionary adaption strategies of tumor cells (33). To date, classical strategies in cancer treatment are based on rapid turnover of cancer cells. Conventional treatments such as chemotherapy, angiogenesis, and immunotherapy have generated mixed results in our quest to conquer cancer. Perhaps shifting focus from cancer cells to cancer microenvironment and host factors—ie, treating the "soil"—could result in more robust results to contain the "seed," ie, the cancer cells.

In theory, limitations of the use of CO_2-saturated solution to achieve tumor hypoxia could include the transient nature of its effect and evolutionary adaptation of heterogeneous cancer cells to an altered environment should treatment be repeated.

In summary, appreciation and understanding of physical properties of tissue that leads to cancer, and the integral role of hypoxia in tumor promotion and progression, not only creates an opportunity for new treatments but also makes us pause and reassess the current practice and therapeutic goals in IO. In middle of every difficulty lies opportunity, and golden opportunity lies in details.

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