# Hyperbaric oxygen: a multifaceted approach in cancer therapy

As one of the fundamental characteristics of solid tumors, hypoxia promotes tumor initiation and progression, induces tumor stemness, and leads to an abundant extracellular matrix (ECM), immunosuppression, and abnormal oxidative stress levels, rendering tumor cells insensitive to treatments such as chemotherapy, radiotherapy, and photodynamic therapy. Therefore, overcoming hypoxia to sensitize cancer therapy has become a current research hotspot. Hyperbaric oxygen (HBO) has emerged as a promising adjuvant therapy in various medical conditions, and there are 14 approved indications for HBO including "Air or gas embolism," "Acute thermal burn injury," "Carbon monoxide poisoning," and to name a few.[1-3] Apart from the listed applications, there are further conditions in which HBO may be useful, which is cancer treatment. HBO delivers pure oxygen with increased atmospheric pressure, which helps tumors overcome hypoxia.

In this perspective, we delve into the multifaceted mechanisms by which HBO could potentially revolutionize cancer treatment (**Figure 1**). Based on our previous works, we summarized four aspects in which HBO could significantly influence cancer therapy: (1) HBO normalizes tumor mechanics and blood vessels by dramatically reducing fibronectin and collagen I within tumor area, which benefits the penetration depth and drug accumulation in solid tumors.[4,5] (2) HBO directly suppresses cancer stem cells (CSCs)[4-7] and cancer metastasis, prominently decreasing both intestinal metastatic nodules in pancreatic cancer mice model and lung metastasis in breast cancer bearing mice.[4,5] (3) HBO could disrupt hypoxia-mediated immunosuppression, which helps programmed death-1 (PD-1) antibody trigger cytotoxic T lymphocytes and long-lasting immunological memory to inhibit tumor recurrences.[8] (4) HBO facilitates an elevation in oxidative stress within cancer cells, working in conjunction with ROS-inducing nanoparticles to promote the accumulation of oxidative damage specifically in cancerous cells.[7,9,10] This process ultimately triggers apoptosis, leading to the demise of cancer cells.

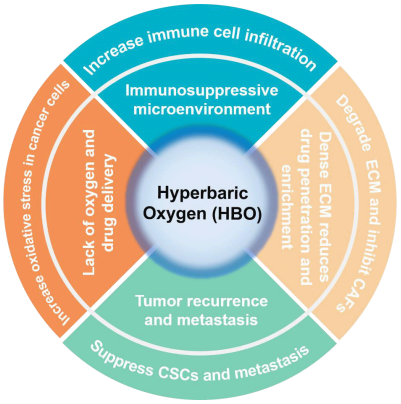


**Figure 1: The mechanisms by which HBO empowers tumor treatment.**
Note: There are four aspects listed above by which HBO boost tumor treatment: (1) Reshape immunosuppressive microenvironment; (2) Suppress ECM and CAF; (3) Eliminate CSCs and reduce metastasis; (4) Increase oxygen concentration and benefit anti-cancer drug therapy. Created with Microsoft PowerPoint 365MSO (version 2308 Build 16.0.16731.20496). CAF: Cancer associated fibroblast; CSC: cancer stem cells; ECM: extracellular matrix.

Taking into account all these aspects, HBO has the potential to collaborate effectively with commercialized nanomedicine or immune checkpoint blockade inhibitors, yielding a range of benefits such as enhanced penetration, increased accumulation in tumor tissues, reduced recurrence rates, and more. This synergy opens up new and promising possibilities in the ongoing fight against cancers.

**Normalizing tumor mechanics and blood vessels through cancer-associated fibroblasts (CAFs) and ECM suppression:** The tumor microenvironment plays a crucial role in tumor progression and cancer therapy. CAFs and ECM surrounding tumors contribute to tumor growth, invasiveness, and the development of abnormal tumor blood vessels. These factors create a hostile tumor microenvironment that hinders drug delivery and immune cell infiltration. HBO has been shown to directly suppress CAFs and remodel the ECM, leading to the normalization of tumor mechanics and blood vessels.[4,5] Additionally, HBO-induced normalization of the ECM can improve the homogeneous distribution of therapeutic agents within the tumor, augmenting their efficacy.[4] HBO regulates the hypoxia-inducible factor 1α/connective tissue growth factor/collagen I pathway in tumors to reduce the deposition of collagen fibrils.[6] On the other hand, HBO can modulate genes associated with collagen biogenesis pathways, such as C-X-C chemokine receptor type 4 and transforming growth factor-β. It also regulates the expression of matrix metalloproteinase 7.[8] Due to the remodeling of the ECM by HBO, reduced solid stress within the tumor contributes to the normalization of tumor vascular structure and function.

Using various techniques, including fluorescent staining of tissue sections, western blotting, quantitative polymerase chain reaction, and flow cytometric analysis, many studies observed significant decreases in several ECM and CAF-related markers such as fibronectin, collagen I, α-smooth muscle actin, and fibroblast activation protein-α in tumors following HBO treatment.[4-6] Additionally, Liu et al.[5] used atomic force microscopy to measure the Young's modulus of tumor tissues before and after HBO treatment, revealing that HBO treatment led to a softening of tumor tissues. Furthermore, Wang et al.[4] observed a reduction in neovascularization, decreased vascular curvature, and increased blood perfusion at tumor tissues by HBO treatment. These experimental results suggest that HBO exerts a positive effect on the mechanical properties and vascular normalization of tumor tissues. Utilizing a 3D tumor spheroid model co-culturing cancer cells with fibroblasts, Wang et al.[4] verified that HBO treatment facilitated deep penetration of Abraxane, providing an explanation for the improved antitumor efficacy when combining HBO with commercialized nanomedicines. These investigations collectively indicate that HBO ameliorates tumor hypoxia and remodels the tumor microenvironment, mechanical properties, and vascular milieu. This leads to an elevation in the delivery efficiency of nanomedicines to tumor sites, encompassing augmented tumor accumulation, enhanced deep penetration, and cellular uptake. Consequently, these effects synergistically contribute to the efficient eradication of tumor stem cells, suppression of tumor metastasis, and heightened antineoplastic efficacy of nanomedicines.

**Direct suppression of CSCs and cancer metastasis:** CSCs are a subpopulation of cells within tumors that possess stem cell-like properties, including self-renewal and tumorigenic capabilities.

Hypoxia and the resultant hypoxia-inducible factor 1α play pivotal roles in initiating and sustaining the stemness of CSCs. These CSCs are highly resistant to conventional therapies and are often responsible for cancer recurrence and metastasis. HBO emerges as a significant modality capable of effectively counteracting the hypoxic conditions prevalent in solid tumors. Through this mechanism, HBO disrupts the maintenance and self-renewal of CSCs, consequently attenuating the stem-like properties within solid tumors. Therefore, HBO has a dual impact on CSCs. First, it diminishes the self-renewal capacity of CSCs, thereby reducing their ability to regenerate. Secondly, HBO enhances the effectiveness of nanomedicines in eliminating CSCs. This synergistic effect is attributed to HBO's improvement of nanomedicine delivery efficiency, alleviation of G0–G1 phase cell cycle arrest, and increased sensitivity to nanomedicine treatments. By decreasing tumor stemness and enhancing stem cell elimination efficiency, HBO significantly suppresses tumor metastasis.[4,6]

In the orthotopic pancreatic cancer mice model, various experimental approaches, including fluorescent staining and flow cytometry analysis of stemness biomarker CD133 within tumor tissues, are employed to verify that HBO-treated groups exhibited significantly lower levels of CD133 expression compared to their untreated counterparts.[4,5,7] This finding suggests that HBO can effectively reduce the stemness of pancreatic cancer cells. Moreover, in the fibrin gel tumor spheroid assay, the authors further validated the reduction of tumor spheroid number and diameter when HBO was combined with several clinical frontline drugs such as Abraxane or Doxil,[4,5] using different tumor cell lines like Panc02 and 4T1 cancer cells. These observations indicate a decrease in tumor cell stemness due to HBO treatment. The same group further investigated both the number of intestinal metastatic nodules in Panc02 tumor models and the lung metastasis in 4T1 breast cancer model, and found that the HBO-treated groups exhibited fewer metastatic lesions compared to their untreated counterparts.[4,5] Taken together, these finding underscore the significance of HBO in modulating the aberrant microenvironment of malignant solid tumors. These findings suggest that HBO has considerable promise in clinical applications for treating matrix- and CSCs-rich solid tumors.

**Disrupting hypoxia-mediated immunosuppression:** Tumor hypoxia is known to create an immunosuppressive microenvironment that hinders the activity of immune cells. The effectiveness of immune checkpoint blockade inhibitors against solid tumors is compromised by the hypoxia induced immunosuppressive microenvironment. HBO has been shown to reverse hypoxia-mediated immunosuppression by promoting the activation and proliferation of various immune cells. Enhanced T-cell and natural Killer cell function, along with increased production of pro-inflammatory cytokines, can lead to improved anti-tumor immunity. Furthermore, HBO's ability to promote dendritic cell maturation and antigen presentation can enhance the adaptive immune response against cancer cells. Liu et al.[8] assessed changes in immune cell infiltration at tumor tissues before and after HBO treatment and found that HBO treatment significantly increased the proportion of lymphocytes, including $CD4^+$ and $CD8^+$ T cells, in the tumor microenvironment, as confirmed by fluorescent staining and flow cytometry of tumor tissues. Based on these results, the authors initiated a clinical trial investigating the combination of HBO with PD-1 antibodies for hepatocellular carcinoma patients.[8] They examined a broad range of immune cell populations and found that HBO, in combination with PD-1 antibodies, resulted in a decrease in myeloid-derived suppressor cells, an increase in the M1/M2 macrophage ratio, enhanced cytotoxic T lymphocyte proportion and proliferation activity, and reduced levels of cytokines like transforming growth factor-β and interleukin-10 in the serum. In the realm of clinical practice, despite emerging as promising anticancer agents, immune checkpoint inhibitors have exhibited limited efficacy against solid tumors. This phenomenon primarily arises due to the immunosuppressive microenvironment and sparse immune cell infiltration. The investigation involving the co-administration of HBO with PD-1 antibodies for the treatment of murine pancreatic cancer underscores the significant capacity of HBO to markedly enhance immune cell, particularly T-cell, infiltration. This effect is shown to reverse the tumor's immunosuppressive microenvironment, thereby offering a conduit for immunotherapeutic interventions and presenting a novel avenue for clinically addressing solid tumors. The synergistic application of immune checkpoint inhibitor agents in conjunction with HBO holds substantial potential for yielding robust anti-tumor effects in clinical contexts.[8]

**HBO helps nanomedicine boost oxidative stress-induced apoptosis in tumor cells:** Cancer cells are sensitive to changes in oxidative stress levels, and elevated levels of oxygen in the presence of HBO lead to an increase in reactive oxygen species (ROS) production within tumor cells. ROS plays a crucial role in inducing apoptosis (programmed cell death) in cancer cells. By elevating oxidative stress, HBO, combined with ROS-inducing nanomedicines, can boost cancer cell apoptosis. This targeted approach holds great potential in developing novel and less toxic cancer therapies.

Many studies developed various nanomedicines and achieved favorable results in treating different types of tumors when combined with HBO.[4-10] For instance, HBO combined with up-conversion nanoparticles photodynamic therapy demonstrated potent therapeutic efficacy in a 4T1 tumor-bearing mouse model.9 Additionally, HBO combined with hydroxyethyl starch-coupled doxorubicin-loaded glucose oxidase nanoparticles depleted glucose within tumor cells, increased oxidative stress, and decreased cell stemness, ultimately inducing tumor cell death through the synergistic actions of chemotherapy, chemodynamic therapy, and starvation therapy.[10] These studies revealed that HBO not only enhanced oxidative stress but also increased drug penetration and accumulation while reducing tumor cell stemness, presenting a multifaceted therapeutic approach.

In a holistic perspective, HBO exhibits a dual functionality within these investigations. Firstly, HBO elevates oxygen tension within solid tumors, ameliorating hypoxic conditions and thereby providing substrates for the generation of ROS. Secondly, HBO treatment remodels the tumor microenvironment, facilitating enhanced penetration and targeted accumulation of nanoparticles. Through these two aspects, HBO significantly augments the cytotoxic impact of nanoparticles utilizing principles such as photodynamic therapy or chemodynamic therapy, wherein ROS are employed for tumor cell eradication. This synergistic enhancement yields robust antineoplastic effects, thereby contributing to a substantial anti-tumor outcome. HBO modulation of tumor oxidative stress levels can guide the design of a new type of ROS-mediated tumor therapy, enabling the development of novel oxygen-dependent nanodrugs. For instance, it is possible to investigate whether HBO can enhance ROS-dependent ferroptosis.

HBO therapy represents a promising approach to complement existing cancer treatments. By targeting multiple aspects of tumor progression, HBO has the potential to suppress CSCs and cancer metastasis, normalize tumor mechanics and blood vessels, disrupt hypoxia-mediated immunosuppression, and sensitize cancer cell through increased oxidative stress. The multifaceted mechanisms of HBO highlight its versatility and capacity to contribute to cancer treatments. As ongoing research continues to elucidate the full extent of HBO's benefits and optimize its integration into cancer therapy, the molecular mechanisms of HBO's effect still have significant gaps. For instance, the mechanisms underlying HBO's reduction of CAFs and ECM, and the specific subtype of CAFs affected, remain to be elucidated. The molecular basis by which HBO inhibits CSCs and the mechanism of selective CSC inhibition while sparing normal cells are still unclear. One of the current research directions is to explore the molecular mechanisms through which HBO inhibits cancer metastasis. Similarly, the precise identity of immune cells and molecules responsible for HBO-induced immune cell infiltration remains elusive. Regarding the changes in oxidative stress, a more comprehensive investigation into the mechanisms is needed, spanning from systemic oxidative stress levels in the whole organism to the impact on the mechanism of mitochondrial oxidative respiration within organelles. Nevertheless, the collective findings summarized in this study underscore the multifaceted efficacy of HBO in the realm of cancer therapy, offering promise in addressing several clinical challenges associated with solid tumors, including suboptimal chemotherapy responses, ineffective immune therapy, tumor metastasis, and relapse. Several ongoing clinical trials are currently recruiting patients, with anticipation for further preclinical investigative outcomes. In summary, HBO's multifaceted mechanisms address critical issues in the treatments of solid tumors, holding significant potential for clinical translation, thereby providing novel insights and opportunities in the landscape of cancer therapy. Finally, it is our hope that this perspective can offer a new direction for gas therapy of cancer and provide a novel approach for clinical management of cancers.

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