Editorial

Can respiratory hyperoxia mitigate adenosine—driven suppression of antitumor immunity?

Peter Vaupel, Arnulf Mayer

Department of Radiooncology and Radiotherapy, Tumor Pathophysiology Section, University Medical Center, 55131 Mainz, Germany
Correspondence to: Prof. Dr. med. Peter Vaupel. Department of Radiooncology and Radiotherapy, University Medical Center, Langenbeckstrasse 1, 55131 Mainz, Germany. Email: vaupel@uni-mainz.de.

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Introduction

Presence of hypoxic sub-volumes, extracellular acidosis, and the accumulation of extracellular adenosine (ADO) and lactate are pathophysiological traits in most human solid tumors (1-3). As a consequence of these properties, tumor cells switch to (mal-)adaptive processes and develop aggressive phenotypes and resistance to treatment [e.g., (4-7)]. In a recent review article, a synopsis was presented of the various mechanisms by which tumor hypoxia *per se* and through indirect actions may contribute to a more aggressive phenotype, to an increased resistance to anticancer therapies, and finally to poor patient outcome (6). In this context it has been outlined that hypoxia-associated ADO-accumulation may be one of the central drivers for the inhibition of anticancer immune response.

There is abundant evidence that the antitumor activities of cytotoxic lymphocytes, natural killer (NK) cells and dendritic cells (DCs) are inhibited in hypoxic tumor microenvironments (TMEs), whereas functions of immune-suppressive cells (Treg cells, tumor-associated M2-macrophages, myeloid derived suppressor cells MDSCs) and immune-suppressive cytokines (e.g., TGF-β, IL-10) are promoted.

Extracellular acidosis in solid tumors also negatively affects cellular and humoral antitumor functions (8,9). Likewise, lactate accumulation can exert a strong inhibitory effect on the antitumor immune-response (10-12).

ADO-rich TMEs are mostly caused by catabolism of extracellular nucleotides (ATP, ADP, AMP) by hypoxia-/HIF-1-sensitive, membrane-associated CD39/CD73-ectoenzymes and signaling through A2A-receptors (13-21). Most probably, extracellular generation is the major source of ADO in the halo of cancer cells upon specific alterations taking place during tumor growth. Intracellular ADO-formation from AMP by a cytosolic AMP-nucleotidase with subsequent ADO-export into the extracellular space through a nucleoside transporter (ENT-1) seems to play a subordinate role.

ADO levels and ADO-receptors in solid malignancies

Extracellular ADO-concentrations in normal tissues have been found to be in the range of 10-100 nM (21,22). In contrast, levels in experimental tumors were reported to be in the μM-range (2,23). In a recent publication, intratumoral ADO levels in severely hypoxic, "high-adenosine" MCA205 fibrosarcomas were in the range of 0.2 to 1.3 μM (21). This level (average: 0.8 μM) was approx. 30 times higher than in the skin overlaying the tumor. These data clearly indicate that tumors—in contrast to normal tissues—accumulate ADO in concentrations high enough to even stimulate the "low-affinity" receptor A2B.

In general, all ADO receptor subtypes A1, A2A, A2B and A3 were found to be up-regulated in various tumor cell lines. Observations concerning the modulation of cancer growth upon activation of A1 receptors are still conflicting (24). A2A-, A2B- and A3- receptors have been implicated in tumor angiogenesis via secretion of pro-angiogenic VEGF, bFGF, angiopoietin-2 and IL-8 (15,24). Notably, A2A-receptors are preferentially involved in the inhibition of the antitumor immune function.

Adenosinergic tumor immune evasion by inhibition of immune cell functions

The seminal observations of Hoskin *et al.* (23,25) have

established a role for ADO in the tumor escape from immune control through blocking antitumor immunity. The function of the A2A-receptor in the adenosinergic inhibition of the antitumor immune action was clearly documented by Ohta and Sitkovsky (26). At present, there is strong evidence that adenosinergic signaling, preferentially through A2A-receptors, in hypoxic, ADO-rich TMEs can lead to a broad spectrum of strong immune-suppressive properties facilitating tumor escape from immune control. These mechanisms include: (I) impaired activity of $CD4^+$ and $CD8^+$ T-cells, of DCs and of cytotoxic NK cells; and (II) activation of Treg cells and expansion of MDSCs, promotion of pro-tumor M2-macrophages and increased activity of the immune-suppressive cytokines TGF-β and IL-10, which directly inhibit T-cell function and promote induction of Treg cells.

Respiratory hyperoxia weakens the hypoxia-driven adenosinergic inhibition of antitumor immune response

Based on the hypoxia-adenosinergic pathway (hypoxia → activation of HIF-1 and CD39/CD73 → ADO accumulation → A2A-receptor stimulation → G-protein- and cAMP-mediated, multifaceted immunosuppression), Hatfield *et al.* (21) have initiated a proof-of-principle study to test whether respiratory hyperoxia (60% O_2) can decrease ADO-levels in the TME in order to improve the antitumor immune response. They could demonstrate that breathing this hyperoxic gas mixture can substantially decrease extracellular ADO levels in severely hypoxic, high-adenosine MCA205 mouse fibrosarcomas by about 75% (estimated from data presented in Figure S5). This drop of the ADO level was the result of a downregulation of hypoxia- and HIF-1α-related genes, including the expression of the ADO-generating CD39/CD73-ectoenzymes, and of COX-2. As a consequence, immune-suppression was "revived", tumor angiogenesis was reduced and the activity of tumor-antigen-expressing molecules was increased, and finally tumor growth was delayed and long-term survival of mice was achieved. These data confirmed the original assumption of the authors that improvement in the tumor oxygenation status can convert the TME from tumor-protecting to tumor-destructing by restoration of the key components of the innate and adaptive immune system involved in antitumor cytotoxicity (e.g., de-inhibition of NK cells and antitumor T-cells). Of note, hyperoxic gas breathing has also been shown to improve clinical outcome of hypoxic tumors treated with accelerated radiotherapy (27).

Hatfield and colleagues have now substantially extended their experiments adding another tumor model and expanding their spectrum of experimental techniques (28). In addition to confirming their earlier observations, they now expand our knowledge with the following key results: (I) inhibition of T-lymphocytes and NK cells is the principle mechanism for anticancer immune-suppression in hypoxic, ADO-rich tumors, triggered by CD39/CD73- and A2A-receptor activation, and cAMP signaling; (II) respiratory hyperoxia promotes tumor regression, decreases lung metastases of experimental breast cancers and prolongs long-term survival in the preclinical setting due to enhancement of tumor infiltration by antitumor $CD8^+$-T-cells; (III) these latter effects are independent of ROS-generation upon respiratory hyperoxia, instead they are entirely established by reactive T-and NK-cells; (IV) severely hypoxic tumor regions show markedly fewer infiltration of antitumor $CD4^+$-and $CD8^+$-cells, thus demonstrating "aversion to hypoxia"; (V) respiratory hyperoxia improves tumor regression in preclinical tumor models of immunotherapies; (VI) expression of cytotoxic T lymphocyte associated protein 4 (CTLA-4) was reduced upon hyperoxic treatment and, perhaps clinically most relevant, rejection of lung metastases following combined CTLA-4 and programmed cell death protein 1 (PD-1) blockade was significantly enhanced by additional inspiratory hyperoxia.

Other mechanisms potentially involved in the reversal of immunosuppressive reactions upon respiratory hyperoxia

From earlier *in-vivo*-studies on experimental tumors there is clear indication that other mechanisms may also be involved in the "rescue" of anticancer immune response using respiratory hyperoxia, especially when considering that normobaric respiratory hyperoxia as used by Hatfield *et al.* (28) can reduce tumor hypoxia only to a limited extent, instead of totally eradicating the O_2-deficiency status (29,30).

Upon respiration of a gas mixture containing 60% O_2 under normobaric conditions, arterial pO_2-values of approx. 350 mmHg will be reached. Under these conditions, only an incomplete improvement in O_2 fluxes can occur that is probably not able to eradicate severe tumor hypoxia in all malignancies, especially human malignancies and considering the multicausal pathogenesis of this condition (31,32). Particularly, abrogation of tissue hypoxia at

the venous end of tumor microvessels is unlikely upon respiratory hyperoxia using 60% O_2 (33,34). This notion is supported by data presented in Figure 1A (right panel) of the previous paper by Hatfield *et al.* (21), which indicates that there still is significant Hypoxyprobe staining upon breathing of 60% O_2.

Other mechanisms possibly involved in the effects now observed by Hatfield *et al.* (28) may include a switch from glycolytic breakdown to a more oxidative metabolism of glucose resulting in a reduced production of lactic acid [–25% (34)] and of protons (H^+-ions), thus significantly attenuating tumor tissue acidosis. Both conditions, accumulation of lactate and acidosis, are known to reduce antitumor immune-suppression, as already mentioned in the Introduction section. Breathing of 60% O_2 may also reduce intracellular ATP hydrolysis through stabilization of the bioenergetic status of cancer cells. As a consequence, production of ADO and protons is reduced. These additional mechanisms upon respiratory hyperoxia may support the anti-adenosinergic processes profoundly discussed by Hatfield *et al.* (28).

Conclusions

With their article, Hatfield *et al.* (28) have significantly expanded current knowledge about the role of ADO as a potent inhibitor of (anticancer) immune-responses, an action that is far beyond its well-known protective functions on the heart, brain and kidneys, in pain management and as an inhibitor of platelet aggregation. Indeed, their novel data have established ADO as a candidate central player of immune suppression mediated by the TME. For their actual research and notable publication Hatfield and colleagues should be highly commended.

Their finding that respiratory hyperoxia reverses the hypoxia-associated adenosinergic immune-escape of malignant tumors is of utmost importance as a rationale for testing supplemental oxygen as a co-adjuvant, either alone or together with already available selective A2A-receptor antagonists and/or CD39/CD73-inhibitors, with existing anticancer immunotherapies. Clinicians should be aware of the fact, that respiratory hyperoxia using 60% O_2 might not be able to completely eradicate all severely hypoxic tumor sub-volumes under normobaric conditions in solid human tumors. Nevertheless, applying respiratory hyperoxia, an easily applicable and thus routinely feasible physical measure, may increase the efficacy of anticancer immune checkpoint therapy (CTLA-4 and PD-1 inhibition) in the clinical setting. Further synergies may arise from the well-established effect of hyperoxic gas breathing during fractionated radiotherapy of hypoxic tumors.

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Footnote

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