



Mild Hyperthermia Induced by Water-Filtered Infrared A Irradiation: A Potent Strategy to Foster Immune Recognition and Anti-Tumor Immune Responses in Superficial Cancers?

G. Multhoff, E. A. Repasky, and Peter Vaupel

10.1 Introduction

Dated back to the 1970s and 1980s, the rationale for the clinical application of hyperthermia (HT) in oncology was to induce direct cytotoxic effects in cancer cells by high, lethal temperatures ($tT \geq 43$ °C). However, this concept was partially abandoned after recognizing that, with the heating technologies available at that time, tumor temperatures ($tT \geq 43$ °C) could not be reached in all tumors/tumor regions treated in the clinical setting [1]. Using the contactless, thermography-controlled water-filtered infrared A (wIRA) irradiation technique which could heat superficial, large-sized tumors, tissue temperatures in the fever-range ($tT = 39$ – 41 °C) or mild hyperthermia levels ($tT = 39$ – 43 °C) up to tissue depths of ≈ 25 mm can be reached. The advantages of this novel technique used in the clinical setting in combination with subsequent hypofractionated radiotherapy have been summarized recently [2–4].

Apart from a number of mechanisms of action as an adjuvant to radio- or chemotherapy, HT also impacts various beneficial effects on anti-tumor immunity. In

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addition to specific effects exerted on various anti-tumor immune responses also related to the overexpression of heat shock proteins (HSPs), HT-induced increases in tumor blood flow and microvascular permeability, and the impact on local delivery of blood-borne immune cells, antibodies, and cytokines will be discussed in this article. HT-induced increases in tumor blood flow, associated with an improvement of the tumor oxygenation status (“reversal of tumor hypoxia”), will be elucidated, based on the fact that tumor hypoxia, a hallmark of cancer growth, is a potent inhibitor of anti-tumor immune mechanisms.

10.2 Mild Hyperthermia Can Enhance the Delivery of Blood-Borne Anti-Tumor Immunity

Mild hyperthermia ($tT = 39\text{--}43\text{ }^{\circ}\text{C}$) can (at least temporarily) increase blood flow in human tumors (for a review, see [5]). In inflammatory, recurrent breast cancer, a frequent medical indication for superficial wIRA-HT, increases in tumor perfusion and hyperemia [i.e., locally increased amount of intravascular blood and O_2 -carrying hemoglobin] outlast the time period needed for the subsequent radiation therapy in the thermo-radiotherapy schedule [6]. In addition to radiosensitization, the increase in blood flow may foster the delivery of blood-borne anti-tumor immunity, i.e., the purposeful accumulation of immune cells, antibodies, and cytokines within the heated tumor mass combined with an upregulation of immunogenic cell surface ligands, such as non-classical (e.g., MICA/B) and classical major histocompatibility complex antigen (MHC) molecules [7].

Intratumor trafficking of anti-tumor immune cells, delivery of antibodies, and anti-tumor cytokines may additionally be supported by hyperthermia-induced increases in microvascular permeability which promotes the diapedesis of immune cells and extravasation of anti-tumor cytokines.

10.3 Mild Hyperthermia Can Attenuate Tumor Hypoxia, a Potent Suppressor of Anti-Tumor Immune Reactions

Tumor hypoxia, i.e., the critically reduced oxygenation of cancer cells and of the TME with oxygen tensions $<10\text{ mmHg}$, is one of the hallmarks of cancers [8]. Tumor hypoxia is a consequence of an impaired oxygen supply due to (a) perfusion-limited delivery, (b) diffusion-limited availability, and (c) hypoxemia (i.e., reduced O_2 transport capacity of the blood in anemic patients or HbCO formation in heavy smokers) [9]. Further details of the various pathogeneses, classifications, time frames, spatial and temporal distributions/heterogeneities of hypoxic subvolumes, variability in the extent (severity) of hypoxia, and consequences of tumor hypoxia have been described elsewhere [10, 11].

Apart from driving malignant progression of primary cancers, firstly described by Höckel and Vaupel, [11–13], hypoxia can lead to acquired resistance in radio- and chemotherapy. Furthermore, hypoxia (and/or upregulation of HIF-1 α) distinctly

impairs immune cells (derived from the TME) exerting effective anti-tumor immune responses (immune hallmark of cancer) [14] while facilitating immunosuppressive cells in terms of their suppressive functions, thus inducing immune tolerance and immune escape (for recent reviews, see [15–19]).

Figure 10.1 schematically illustrates the effects of tumor hypoxia on key immune cell populations. In general, hypoxia negatively impacts the survival and functions of antigen-presenting cells (APCs) and effector cells and decreases the release of effector and proliferative cytokines [e.g., interferon- γ (IFN- γ), interleukin 2 (IL-2)],

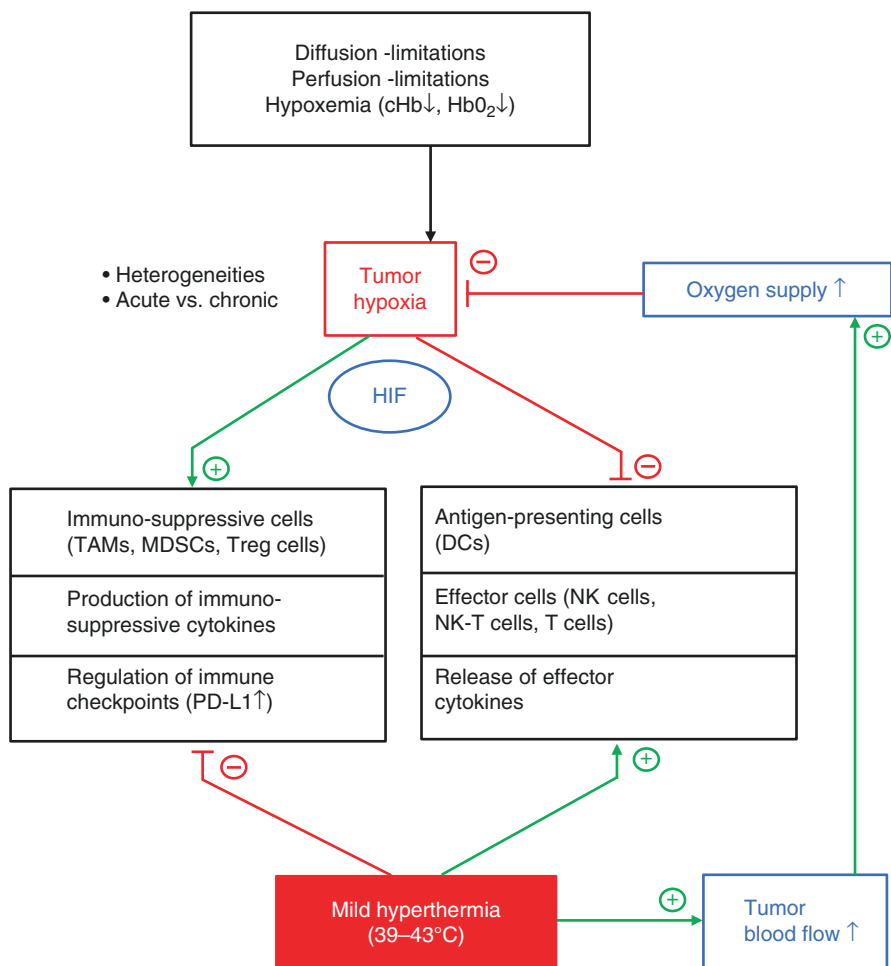


Fig. 10.1 Simplified flow chart describing the supportive impact of tumor hypoxia (with pathogeneses and some characteristics) and HIF-1 α on immunosuppressive measures (left), and compromising effects on anti-tumor immunity (right). Mild hyperthermia with tumor temperatures of 39 °C–43 °C can lead to a reversal of tumor hypoxia through improvements in the oxygen supply triggered by increased tumor blood flow upon tissue heating

while supporting immunosuppressive cells and promoting the production of immunosuppressive cytokines (e.g., transforming growth factor- β). Besides these mechanisms, several immune checkpoint molecules are regulated by hypoxia (e.g., PD-L1) and thus contribute to immune evasion. Tumor hypoxia can be reversed by mild hyperthermia ($tT = 39\text{--}43\text{ }^{\circ}\text{C}$) via heat-induced increases in tumor blood flow and, in turn, enhanced oxygen supply, as shown in this simplified flowchart.

In more detail, hypoxia (and upregulated, stabilized HIF-1 α) dampens anti-tumor immune responses by (a) reducing the survival as well as the cytolytic and migratory activities of *effector cells* [e.g., CD4⁺ T helper cells, CD8⁺ cytotoxic T cells, natural killer-like (NK-T) cells, and natural killer (NK) cells; Fig. 10.2a]; (b) reducing the production and release of *effector cytokines* [e.g., granzyme B, perforin, IFN- γ]; (c) impairing the differentiation and function of *APCs* [e.g., dendritic

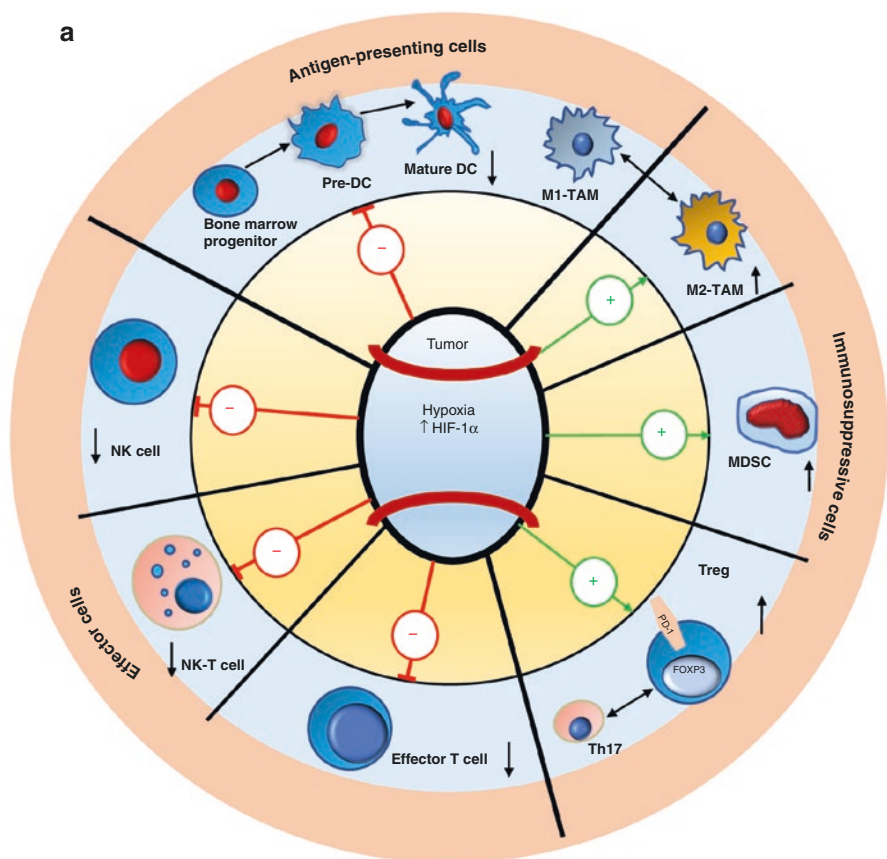


Fig. 10.2 Detailed mechanisms of hypoxia- (HIF-1 α -) induced immunosuppression in cancers (a), and improved anti-tumor immune responses upon reversal of tumor hypoxia triggered by mild hyperthermia, HT (b). Green arrows: activation, red T-bars: inhibition. *TBF* tumor blood flow

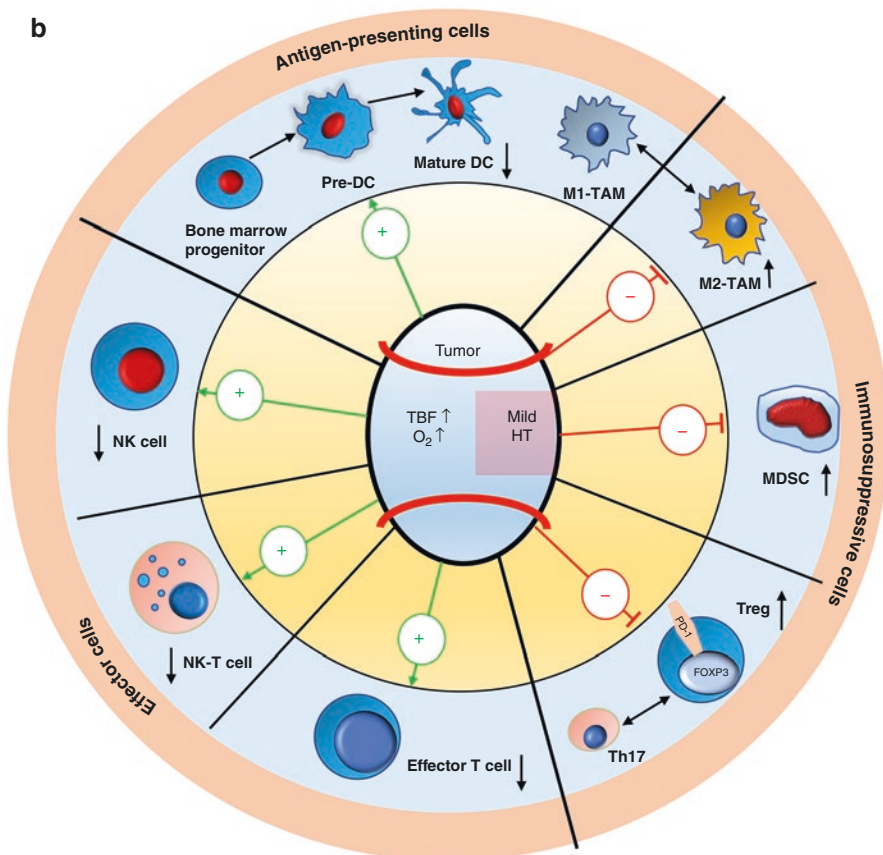


Fig. 10.2 (continued)

cells (DCs), Langerhans cells in the epidermis of the skin]; (d) driving *immunosuppressive cells* [e.g., regulatory T (Treg) cells, myeloid-derived suppressor cells (MDSCs) and tumor-associated M2-macrophages (TAMs)]; (e) increasing the production and release of *immunosuppressive cytokines* (e.g., IL-10); and (f) upregulating of the expression of *immune checkpoint inhibitors* (e.g., PD-L1, [19]).

10.4 Metabolic Reprogramming Impacts Anti-Tumor Immune Responses: Role of Mild Hyperthermia?

Another hallmark of cancer cells is metabolic reprogramming [8]. A major metabolic pathway of this phenotype is aerobic glycolysis (Warburg effect), which is characterized—inter alia—by a high lactate⁻ and H⁺ (proton) output/export into the TME [20, 21], finally leading to an extracellular lactate accumulation (up to 40 mM) and tissue acidosis (pH <6.8). Both conditions constitute major inhibitors of

anti-tumor immunity, i.e., cancer cells utilize this detrimental microenvironment to escape from anti-tumor immunity. Acidification of the microenvironment and high lactate⁻ levels can thwart anti-tumor immune responses by (a) compromising, e.g., the proliferation and activity of CD8⁺ and CD4⁺ T cells, DCs, NK and NK-T cells, and the release of immuno-stimulatory TH-1 type cytokines, and by (b) activating the immuno-suppressive effects of Treg cells, MDSCs, and M2 macrophages, increasing the expression of immune checkpoints inhibitors and promoting the release of TH-2 type cytokines [22, 23].

Based on current knowledge, shaping anti-tumor immune responses by targeting metabolic reprogramming or signaling pathways using mild hyperthermia (tT = 39–43 °C, 60 min) has not been investigated so far. Earlier experiments using fast-growing rat tumor isografts exposed to localized hyperthermia (tT = 43.4 °C for 120 min) led to a reduction of the laser Doppler flow rate of 18%, a minimal drop of the average pO₂ values ($\Delta pO_2 = -1$ mmHg), a decrease in mean pH ($\Delta pH = -0.21$ units), and an increase in the mean tissue lactate⁻ concentrations ($\Delta C = +8$ mM) [24, 25]. These findings question the role of mild-to-moderate hyperthermia for 2 h on the Warburg effect and its impact on anti-tumor immune responses, at least in this experimental setting.

10.5 Mild Hyperthermia Augments the Synthesis of Heat Shock Proteins (HSPs) and Increases Tumor Antigenicity

10.5.1 Heat Shock Proteins (HSPs) in Normal and Tumor Cells

Mild hyperthermia (tT = 39–43 °C) in combination with X-ray irradiation increases the formation of reactive oxygen species (ROS), promotes genomic instability, and impairs the DNA double-strand break repair [26]. Moreover, fever-like temperatures interfere with pathways involved in cell cycle regulation and proliferation and can cause protein denaturation and aggregation. Therefore, after exposure of cells to heat generally reduces the synthesis of proteins, apart from that of a special class of proteins, termed heat shock (HSPs) or stress proteins, which consist of at least one ATPase domain and a substrate-binding domain. Apart from thermal stress, their synthesis is also strongly upregulated upon a large variety of different environmental stress factors including changes in oxygen supply, pH, nutrient deficiency, heavy metals, ethanol, radiation (UV, ionizing), cytostatic drugs, hypoxia, and re-oxygenation, etc. [27]. HSPs maintain protein homeostasis, assist protein transport under physiological conditions (e.g., cell proliferation, differentiation, maturation, antigen presentation), and protect cells from lethal damages induced by stress. The biological relevance of HSPs is documented by their ubiquitous distribution, high abundance, and conserved sequence homology in prokaryotic as well as eukaryotic cells.

Normal and tumor cells differ significantly in their proliferative capacity and metabolic demand which is closely related to molecular features of the Warburg effect [21]. Therefore, rapidly proliferating tumor cells generally exhibit a cytosolic

overexpression of HSPs which are localized in nearly all subcellular compartments including cytosol, nucleus, endoplasmic reticulum, lysosomes, endosomes, and mitochondria [28]. Following environmental stress including elevated temperatures, hypoxia, chemotherapy, and radiotherapy, the synthesis of all HSPs and especially that of the major stress-inducible Hsp70 is further upregulated to prevent tumor cells from stress-induced lethal damages including protein misfolding, denaturation and aggregation, and transport deficiencies [29, 30]. In contrast to normal cells, viable tumor cells present a number of HSPs, including Hsp70 on their plasma membrane [31, 32] via a tumor-specific glycosphingolipid anchorage [33], and actively release HSPs in lipid micro-vesicles termed exosomes [34].

10.5.2 Role of HSPs in NK and T-Cell-Mediated Immunity

Depending on their intracellular, membrane, or extracellular localization, HSPs fulfill different tasks. High cytosolic and mitochondrial HSPs increase the tumorigenic and metastatic potential of tumor cells and prevent apoptosis, whereas membrane-bound and extracellular HSPs [35, 36] have been identified as potent stimulators of the adaptive and innate anti-cancer immune responses. Following cross-presentation of HSP-chaperoned immunogenic peptides on MHC class I molecules, HSPs support CD8⁺ cytotoxic T-cell responses [37, 38] with Toll-like, scavenger, and C-type lectin receptors playing pivotal roles in mediating the uptake of HSP-chaperoned peptides [39, 40]. In the absence of immunogenic peptides, Hsp70 stimulates the cytolytic activity of NK cells in a pro-inflammatory (e.g., IL-2, IFN- γ) environment against membrane Hsp70-positive tumor cells. A phase I clinical trial revealed an excellent safety profile of adoptively transferred, ex vivo Hsp70-activated NK cells [41], and favorable clinical responses in a phase II clinical study in patients with advanced NSCLC after radio-chemotherapy [42]. In line with these findings, a mild heat treatment of tumor cells (including chondro-, osteosarcoma, and liposarcoma) cells increases the membrane Hsp70 density on tumor cells and thereby enhances their susceptibility to NK cell-mediated killing [43–45]. In a preclinical colon carcinoma model, the beneficial effects of a unilateral applied hyperthermia could be associated with an increased CD4⁺/CD8⁺ T and NK cell activity in mice with bilateral tumors, which indicates that local heat to one tumor site has the capacity to induce abscopal effects [46, 47]. Moreover, local hyperthermia used in combination with radiotherapy and/or chemotherapy [48–50] enhances the efficacy of both therapeutic concepts and boosts protective anti-cancer immune responses mediated by CD8⁺ T, NK-T, and NK effector cells.

10.6 Conclusion

Mild hyperthermia ($tT = 39\text{--}43\text{ }^{\circ}\text{C}$) increases tumor blood flow and microvascular permeability, the trafficking of blood-borne immune cells, and the delivery of antibodies and cytokines. In addition, HT-related increases in tumor blood flow are also

associated with an improvement of the tumor oxygenation status (“reversal of tumor hypoxia”) which in turn attenuates hypoxia. The complexity of molecular pathways that coordinate a response to heat is mirrored by diverse cell types that are affected by hyperthermia including DCs, M1 macrophages, effector T, NK-T and NK cells, and immunosuppressive TAMs (M2 macrophages), MDSCs and Treg cells, cytokines, and chemokines (Fig. 10.2b). The majority of data support the hypothesis that temperature shifts in immune cells that may be associated with the application of localized hyperthermia in cancer therapy could promote long-term protection against tumor growth via the recruitment of several mechanisms of the immune system. Furthermore, mechanistic insight into the immune-protective nature of mild hyperthermia has revealed new avenues to exploit the immunostimulatory activities of thermal stress in the context of cancer therapy.

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