

## Hyperthermia has Consistently Improved the Efficacy of Radiotherapy and Chemotherapy for Many Types of Cancers

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### Abstract

Cancer stem-like cells (CSCs) are a subset of cancer cells that are resistant to conventional radiotherapy and chemotherapy. As such, CSCs have been recognized as playing a large role in tumor initiation and recurrence. Although hyperthermia is broadly used in cancer treatment either alone or in combination with radio- or chemo-therapy, its potential to target CSCs is not well understood. In this review, we discuss different types of hyperthermia and potential mechanisms of action in cancer treatment, particularly in regards to killing CSCs.

**Keywords:** hyperthermia; cancer Stem-like cells; chemotherapy; cytotoxic treatment; radiation; nanoparticle; ablation; LITT

### Introduction

Despite advances in understanding the molecular changes underpinning cancer and improved technology and treatments, cancer remains a leading cause of death in America. The cancer stem-like cell (CSC) hypothesis posits that a subset of tumor cells have a high capacity for self-renewal, have the ability to differentiate into multiple lineages and can give rise to tumors [1-4]. These CSCs are highly malignant and can persist or proliferate in spite of cytotoxic treatment [1-4]. Therefore, these CSCs play a large role in tumor progression. Development of new treatment modalities that are able to target and kill CSCs may provide more durable cancer control [1-4].

Hyperthermia is a potent radiosensitizer that has been shown in numerous clinical trials to improve tumor control. Importantly, the efficacy of hyperthermia is seen across many cancer types, including breast cancer, prostate cancer, melanoma, sarcoma, rectal cancer, bladder cancer, esophageal cancer, cervical cancer and glioblastoma suggesting that it has broad clinical applicability [5–24]. Recently, combined hyperthermia and radiation has also been shown to improve pain palliation in patients with bone metastases compared to radiation alone [25]. Therefore, hyperthermia has widespread usage for patients with both locoregional disease and advanced cancers and can be used for patients with a variety of cancer types. The value of hyperthermia as a treatment has in fact been observed for centuries. Hippocrates, the father of modern medicine, is known to have said, “Those who cannot be cured by medicine can be cured by surgery. Those who cannot be cured by surgery can be cured by heat. Those who cannot be cured by heat, they are indeed incurable”. Over the years, medicine and surgery have seen significant advances, and hyperthermia fell by the wayside. However, in modern times, hyperthermia is making a resurgence due to improved technology in delivering hyperthermia and in non-invasive thermometry techniques.

Hyperthermia is classified into two broad categories based on the target heating temperature. Thermal ablation refers to treatments with target temperatures above 50°C and mild temperature hyperthermia refers to treatments with temperatures between 39 and 43°C [26]. While thermal ablation largely kills tumor cells due to the direct cytotoxic effects of heat, mild temperature hyperthermia uses heat as an adjunct treatment to enhance the cytotoxic effects of radiation and chemotherapy [26–28]. The biologic effects of thermal therapy are dependent on time and temperature.

The mechanisms underlying the biologic effects are multi-factorial and impact the tumor population itself, the tumor microenvironment and immune system.

### Methods for Administering Hyperthermia

Radio-frequency hyperthermia is the most widely used hyperthermia technique worldwide and is typically used for ablative heating [28–30]. To achieve heating, radio-frequency electrodes are passed into the tumor tissue under image guidance. A high-frequency alternating current is then passed through the electrodes to cause the rapid oscillation of ions in nearby cells, resulting in frictional heating [27,31]. The range of heating is limited to the millimeter range because it relies on heated tissue to conduct current to surrounding areas [32]. The short range of heating also limits the ability to heat tumors near blood vessels because the heat is dissipated too quickly [32,33].

Microwave hyperthermia is an alternate method of delivery that can overcome some of the limitations of radio-frequency hyperthermia. Microwave heating uses waves of higher frequency to kill cells. Unlike radio-frequency thermal therapy, microwave hyperthermia does not pass an electrical current through tissue, but rather creates an oscillating electromagnetic field that forces ions and dipoles to align with the field, causing them to rotate as the field oscillates [31,32,34]. This rotation causes friction that heats the tissue. Microwave hyperthermia presents several advantages compared to radio-frequency hyperthermia. While radio-frequency hyperthermia relies on ions inside tissue to conduct current, microwave hyperthermia creates an electric field, the effective range of which is larger without risking damage to tissue closer to the antenna or probe [32]. Microwave hyperthermia has a much higher effective range of up to 3 cm [32].

Laser interstitial thermal therapy (LITT) is a relatively new method of administering hyperthermia that uses a stereotactically placed laser probe to heat surrounding tissue with a low power (10–15 Watts) infrared laser (at Nd-YAG range) [35,36]. Heat essentially is produced after absorption of laser in the tissue and transferred up to 1.5–2 cm from the laser probe by conduction. To control the extent of thermal ablation, a specific sequence of MRI (MR-thermometry) is used to measure relative changes of temperature within the magnetic field. For deep seated lesions, including brain tumors, LITT is used in conjunction with MR-thermometry to give accurate thermal ablation of the target lesion [35,36].

The minimally invasive nature of LITT under MR-thermometry guidance has permitted the expansion of hyperthermia to deep and difficult to access tumors including intracranial and retroperitoneal tumors [35,36].

High intensity focused ultrasound (HIFU) (also called focused ultrasound surgery (FUS)) utilizes an ultrasound beam with very high energy to increase the temperature rapidly in the target tissues [37–39]. A single HIFU exposure usually treats a very small volume along the ultrasound axis. Multiple exposures can be used side by side to achieve coverage of a large volume of tumor [37–39]. One advantage of HIFU is that it creates a steep temperature gradient in a small focused area and effectively creates a sharp boundary of damage in the target tissue while sparing adjacent normal tissues [37–39].

Nanoparticles can also be used to augment heating within a tissue when exposed to electromagnetic energy [40–42]. These particles include magnetic nanoparticles (such as iron oxide), gold-silica nanoshells, solid gold nanoparticles and carbon nanotubes [40,41]. The outer shell of nanoparticles can be modified molecularly to facilitate their dissemination and uptake by specific cell types, including tumor cells [42]. Additionally, nanoparticles may be loaded with cargo including cytotoxic drugs or oncolytic viruses that can be released upon disruption by a heat source [43]. Nanoparticles can be administered systemically to exploit the leaky vasculature of primary tumors to enhance intra-tumoral delivery [44]. However, nanoparticles often display a patchy, near-perivascular deposition within the well-vascularized regions of tumors [45]. Some blood vessels such as those associated with brain tumors are not as leaky as blood vessels found in other solid tumors. While the blood-brain barrier is partially breached in regions with glioma cells, the ‘compromised’ blood-brain barrier still presents a major challenge, especially in hypoxic and avascular region of glioma dispersion [46]. Since high spatial concentrations of nanoparticles are required for hyperthermia, direct intratumoral delivery of 12 nm magnetic nanoparticles has been used in clinical trials for hyperthermia treatment of prostate tumors and recurrent glioblastoma [47,48]. When subjected to an external alternating magnetic field, the nanoparticles vibrate and heat up to kill surrounding cells. Because non-ionizing electromagnetic radiation can be applied remotely to heat the nanoparticles, this technology is considered noninvasive but requires good visualization of the target tumor [42,49]. A typical drawback of the application of iron oxide nanoparticles is associated with the indefinite exclusion of MRI for subsequent monitoring of tumor progression after initial injection of nanoparticles and the residual MR signals that interfere with follow-up MR imaging.

### Hyperthermia in Cancer Therapy

When hyperthermia is applied to a tumor, three different reaction zones can be distinguished based on the temperature and duration of heating: a central zone that is directly and immediately beyond the application site, a peripheral zone that is around the central zone and is heated to a lower temperature, and an outer region which is not directly affected by the heat [28,42,50]. Hyperthermia causes cellular injury directly and indirectly in these different zones via different mechanisms, although some overlap may exist. The extent and type of cellular damage varies as a function of temperature and time. A high temperature for a short period of time can achieve similar levels of cell kill as lower temperature heating for a longer period of time.

**Direct Effects to Tumor Cells:** Hyperthermia causes membrane dysfunction to contribute to cell death. Rising temperature affects the stability, fluidity and permeability of cellular membranes, including the plasma membrane, mitochondrial membranes, and other cytosolic membranes [50,51]. These membrane changes can compromise the function of transmembrane transport proteins, ion channels, cell surface receptors and other membrane-associated proteins and disrupt lipid rafts or signal transduction hubs [50,51]. However, the degree of membrane dysfunction strongly influences cell fate. For example, some changes in membrane potential, intracellular sodium and calcium content do not correlate well with the rate of cell death [52–54].

On the other hand, mitochondrial dysfunction induced by hyperthermia can lead to cell death [50,55].

Another direct effect of heat is the denaturation of proteins, especially under high temperatures. Denaturation and inactivation of these proteins can impact a broad range of cellular processes including cellular metabolism, protein synthesis, nucleic acid synthesis and DNA/RNA polymerization [50,56]. After mild hyperthermia, some cellular functions can recover. Proteins may refold, and RNA and protein synthesis may recover. However, DNA replication and repair typically remain repressed [57]. This is thought to be due to the aggregation of denatured proteins in the nuclear matrix and irreversible changes to chromatin structure that impair DNA synthesis and repair [57]. Hyperthermia can inhibit the function of DNA-polymerases- $\alpha$  and  $\beta$  and can also facilitate degradation of the DNA repair protein BRCA2 to inhibit homologous recombination [57,58]. Hyperthermia itself is believed not to cause severe DNA damage, but rather indirectly contributes to DNA damage by reducing the efficiency of the DNA damage repair machinery [59].

Hyperthermia can induce cell death by necrosis and apoptosis. The cells in central application zones, which are confronted with high temperatures, usually die by necrosis. However, some subpopulations of cells may escape immediate hyperthermic killing. These resistant cells in the central zone and the cells in the peripheral zone, which receive lower temperature hyperthermia, may die within hours of heat cessation [50,51]. Mild temperature hyperthermia can induce apoptosis through both the intrinsic and extrinsic pathways. Hyperthermia can activate Caspase-2 which then binds to the adaptor protein RAIDD to cleave and activate Bim, which promotes mitochondria-dependent apoptosis [60]. Hyperthermia can also activate Bim to induce Caspase-2 independent apoptosis. In addition, hyperthermia can activate Fas, tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) to trigger the extrinsic apoptotic pathway [61,62]. Additionally, heat treatment can increase the production of reactive oxygen species (ROS) by activation of xanthine oxidase and/or facilitating mitochondria respiration to produce  $O_2^-$ . Moreover, hyperthermia negatively affects SOD1 expression and the enzymatic activities of SOD1 and SOD2, whereas it is able to activate NADPH oxidase [63–65]. This increase in ROS can facilitate apoptosis [66,67].

### Alterations to the Tumor Microenvironment

Hyperthermia can also modify the tumor microenvironment to modulate tumor growth and recurrence. Hyperthermia is well known to increase perfusion within tissue and compromise the integrity of blood vessels [33,68–70]. Hyperthermia can damage endothelial cells, alter the adhesiveness of the vessel wall, and increase the leakiness of blood vessels and viscosity of blood [68]. These changes in perfusion can influence local pH, oxygen and nutrient supply in the tumor, rendering them more stressed and more susceptible to cytotoxic therapy [71]. Hyperthermia can also improve tumor oxygenation, making cells more susceptible to radiation, and may improve the penetration of chemotherapy into the tumors [69,72].

### The Effects of Hyperthermia on Cancer Stem-Like Cells

Cancer stem-like cells (CSCs) are a rare population of cancer cells that can self-renew and differentiate into progeny with limited proliferative potential. CSCs sit at the apex of hierarchically organized tumors. CSCs have strong tumorigenic activity compared to non-stem cancer cells and can establish an entire tumor [1,3,56]. CSCs usually reside in specific niches that orchestrate their fate. Niche components that support the undifferentiated state of CSCs include communication with contacting cells such as other stromal cells and endothelial cells, extracellular matrix components, soluble factors including Wnt, TGF $\beta$  and other cytokines, and physical states such as hypoxia and low pH [73,74].

### Conclusion

The use of hyperthermia to treat cancer has a long history. Hyperthermia has consistently improved the efficacy of radiotherapy and chemotherapy for many types of cancers. The CSC model sheds light on another potential therapeutic benefit of hyperthermia.

Strategies that combine hyperthermia with cytotoxic agents, metabolic stressors or immune therapies may improve CSC kill by targeting the cancer cells themselves and modulating their microenvironment. The method of administering heat may also influence cell kill. More work is needed to define the optimal modes of hyperthermia to kill CSCs safely and efficiently. The combination of hyperthermia and immunotherapy to target CSCs also holds great potential, and further studies are needed to understand how best to integrate hyperthermia with immuno-oncology. Heat therapy was recognized for its therapeutic effects by ancient physicians. It is once again emerging as an important treatment modality that fights cancer through multiple mechanisms.

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