# Thermo-chemotherapy; Magnetic Hyperthermia and 5-fluorouracil

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Limitations of current cancer therapies require more effective therapeutic strategies. Single-modality therapies such as chemotherapy or radiotherapy are not efficient enough to overcome complicated forms of cancer. Conversely, multimodal approaches like combinatorial hyperthermia and chemotherapy have shown promising therapeutic results. Multifunctional magnetic nanoparticles (MNPs) enable the application of local magnetic hyperthermia and the delivery of chemotherapeutics into tumors. This study demonstrates the potential of using MNPs for the application of a combination of magnetic hyperthermia and 5-fluorouracil-based chemotherapy to treat colorectal cancer in tumor-bearing mouse models.

Keywords: 5-fluorouracil ; magnetic nanoparticle ; colorectal cancer ; magnetic hyperthermia

## . Introduction

Colorectal cancer is the third most common and second deadliest cancer worldwide, which led to approximately 881,000 deaths in 2018[1]. Colorectal cancers mostly develop from non-cancerous polyps over a period of 10 to 15 years[2]. Particularly in earlier stages and subsequent to adjuvant therapy, such as chemotherapy and radiation therapy, surgery is one of the standard treatments for this cancer entity. Among the many available chemotherapeutic drugs, 5-fluorouracil (5FU) is widely used. Namely, 5FU is an analogue of uracil that rapidly enters the cell and converts itself into active metabolites, such as fluorodeoxyuridine monophosphates, fluorodeoxyuridine triphosphates, and fluorouridine triphosphates[3]. These intracellularly active 5FU metabolites inhibit the activity of thymidylate synthase and interfere with RNA and DNA synthesis. Thymidylate synthase is an important enzyme for pyrimidine synthesis, and pyrimidine is crucial for DNA replication. Unfortunately, a monotherapy of colorectal cancer with 5FU is not highly effective (approximately 10–20% response rates), and an increase in dose would lead to adverse side effects and drug resistance[4]. Interestingly, the combination of 5FU with other chemotherapeutic drugs, such as oxaliplatin, has improved the tumor therapeutic outcomes[5]. However, further therapeutic strategy developments seem to be urgently needed in order to decrease adverse side effects during treatment.

Combining hyperthermia and chemotherapy as a therapy option has recently gained the attention of researchers and oncologists. In cancer therapy, hyperthermia is defined to be the treatment of a tumor with a temporary rise of temperatures (≥43 °C, 60 min for oncological purposes)[6][7]. Basically, the application of the required temperatures can be performed locally, regionally, and in the whole body using different techniques[8]. Previous studies have confirmed that a temperature increase up to 39 and 42 °C for 30 to 360 min leads to tumor cell death by necrosis and/or apoptosis[6][9][10]. With particular consideration of colon carcinomas, mild hyperthermia tumor treatments (e.g., 41.5 °C for 2 h) have been reported to significantly delay tumor growth[9]. It has further been shown that hyperthermia has an impact on the cytotoxic potential of various anti-cancer drugs, due to an effect called “thermal chemosensitization”[6].

Combining regional radiofrequency-based hyperthermia, which has an external heat source, with radiotherapy and chemotherapy to treat colorectal cancer has shown promising therapeutic results[11]. However, when using an external heat source, it is challenging and it requires adequate tools to properly focus the delivered energy and raise the temperature only on the target tumor tissue. On the contrary, internal heating sources have the advantage of facilitating a highly localized energy deposition within the tumor region. In this respect, and with regard to the combination of hyperthermia with standard oncological treatment modalities, magnetic hyperthermia has recently become increasingly the focus of research. In principle, tissue internal heating sources can be deposited by utilization of various magnetic materials, such as iron oxide magnetic nanoparticles (MNP) [12]. Such materials can be deposited by injection into tumors. They transiently generate heating when exposed to an alternating magnetic field (AMF)[13]. MNPs used in magnetic hyperthermia applications typically have a single or multi-domain magnetite core (e.g., iron oxide) coated with an organic and/or inorganic material. The appropriate selection of MNPs with a high specific absorption rate (SAR) to generate the

required heat and their potential for coupling them with anticancer drugs for local delivery are key factors to effectively kill proliferating tumor cells[6][14]. Several studies used different combinations of magnetic hyperthermia with various chemotherapeutic agents (e.g., doxorubicin and methotrexate) after having attached them to the coating material (mostly a polymer) on the surface of MNP for the treatment of various cancers in mice, such as breast and bladder [15][16].

Interestingly, several studies used chitosan-coated MNPs (CS-MNPs) to encapsulate 5FU for drug delivery applications as a single therapeutic modality[17][18] or in combination with hyperthermia[19][20]. Further on, it has been reported that the combination of 5FU with magnetic hyperthermia leads to a reduced viability and proliferation behavior of colon cancer cells in vitro[19][20]. However, since these studies were carried out mostly in vitro as proof of principles, further in vivo investigations are essential.

## 2. More Efficient Treatment of Heterotopic Human Colon Xenograft Tumors

Authors finally demonstrated that the thermo-chemotherapeutic treatment caused a persistent reduction of endothelial cell marker CD31 protein expression with increasing time after therapy compared to the either therapies alone. CD31 is a marker for endothelial cells and reflects the development and distribution of vessels in different parts of the body as well as in tumors[21]. Moreover, the combinatorial tumor treatment caused a sustained reduction of the proliferation marker αvβ3 integrin in tumor endothelial cells after therapy. In contrast, presence of αvβ3 integrin in the untreated tumors was steadily increasing. αvβ3 integrin is a transmembrane receptor, which is overexpressed on tumor endothelial cells and was reported to be associated with angiogenesis in cancer[22]. This means that the combinatorial treatment was able to damage the tumor vascular system, which could be due to a synergistic effect of the combination of heat and 5FU; this is another therapeutic effect that surpasses the combinatorial treatment over the either therapies alone. It is reported that 5FU, not only kills tumor cells, but is also toxic to endothelial cells and inhibits VEGF-induced angiogenesis in tumor[23][24]. An impairment of the tumor vascular system is beneficial for anti-tumor therapies, since it leads to a restricted supply of nutrients and oxygen due to reduced blood flow. Beyond the influence of thermo-chemotherapy on tumor vasculature, heat could affect the components of the extracellular matrix as well, particularly the collagen fibers[25]. Our group recently reported that mild iron oxide MNP-based hyperthermia (40 °C, 42 °C, 60 min) considerably reduces the integrity of collagen fibers in heterotumor spheroids composed of pancreatic cancer cells and fibroblasts compared to control collagen fibers held at 37 °C[28]. Other studies have shown the same effect of temperature rise on collagen architecture in extracellular matrix of epidermoid carcinoma tumors in vivo[26][27]. These findings imply that if there is some degree of desmoplasia in our heterotopic colon tumors, heat may damage the collagen fibers in their extracellular matrix. However, more detailed understanding of the impact of combination of local hyperthermia and chemotherapy on extracellular matrix and collagen fibers requires comprehensive investigations.

In this study, authors demonstrated that the treatment of colorectal cancer with the thermo-chemotherapeutic treatment composed of magnetic hyperthermia and 5FU-MNP-based chemotherapy was much more effective and led to a better therapeutic outcome in tumor bearing mice than magnetic hyperthermia or 5FU-MNP-based chemotherapy alone, as reflected by the distinctly reduced tumor volume and inhibited tumor cell proliferation (i.e., Ki67 protein expression). Authors could further show that the combinatorial treatment induced extensive DNA damages and disrupted of DNA repair processes in heterotopic colon tumors, which led to extensive cell death shortly after the second tumor therapy. The unchanged or only slightly decreased expression of c-PARP, caspase-3, caspase-8 and c-caspase-8 indicate that the classical caspase-dependent apoptosis was not the leading pathway of cell death after the combined thermo-chemotherapeutic treatment. No sign for necroptosis could be detected as well. Authors hypothesize that the presence of ROS plays distinct role in post-therapeutic cell responses, which prospectively leads, at least in parts, to cell death via oxeiptosis. Additionally, tumor cells surviving the thermo-chemotherapeutic treatment showed several stress responses, such as late overexpression of p53. Moreover, the decreased or unaltered expression of HSP70 and HSP90, PARP, and NF-κB prospectively renders treated tumor cells more sensitive to further therapy-based stress conditions. The thermo-chemotherapeutic treatment finally led to a distinct impairment of tumor vasculature. For these reasons, a very effective elimination of colon cancers seems to be feasible by utilization of repeated thermo-chemotherapeutic therapy sessions in the long-term.

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