Vascular-focused hyperthermia using gold nanoshells enhances radiotherapy efficacy

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By raising the temperature in tumors, optically activated nanoparticles can make existing treatment more powerful.

Heat has been used as an anti-tumor therapy for centuries. One such treatment, mild temperature hyperthermia, does not directly eradicate tumors but sensitizes them to other treatments like radiotherapy. Despite growing evidence of its effectiveness, clinicians often avoid the approach because the methods to attain, maintain, and monitor hyperthermia are unwieldy and invasive. To get around these problems, we have developed a strategy combining optically activated nanoparticles and magnetic resonance imaging (MRI) to noninvasively induce and monitor mild temperature hyperthermia.

Nanoshells (∼150nm) with a silica core and a gold shell are biologically inert and can be optically activated. By varying the ratio of core size to shell thickness, they can be tuned to maximally absorb and convert near infrared (NIR) light to heat. NIR light has the most clinical utility because its low attenuation allows for deeper penetration of tissue. When administered intravenously, the nanoshells easily leak through immature tumor blood vessels to accumulate preferentially within the growths. The particles accumulate passively, then generate heat upon NIR illumination.

First, we demonstrated that we could noninvasively and reproducibly achieve mild temperature hyperthermia. We directly inserted thermocouples into the center and base of nanoshell-filled colorectal cancers that were artificially created on mouse thighs. Next, we established optimal laser parameters with gradual elevation of core tumor temperature to mild hyperthermia levels (∼41°C) for ∼20min. These direct measurements were reconfirmed using MRI thermometry, a real-time noninvasive means of monitoring hyperthermia (see Figure 1).

Next, we evaluated the time to tumor volume doubling in mice treated with radiotherapy—both with and without hyperthermia immediately preceding it. Tumors took nearly twice as long to double in volume after combined treatment as compared to radiotherapy alone. We wanted to understand the enhanced efficacy of combined therapy, given that mild temperature increases alone do not kill cancer cells. Therefore, we first looked at hyperthermia’s effect on blood flow to the growths. Dynamic contrast enhanced MRI (DCE-MRI) showed that immediately after raising temperatures (<5min), blood flow to the core of the tumor significantly increased. This higher flow of blood oxygenated the tumor’s central regions that are normally deficient in oxygen (see Figure 2). Reducing the number of these hypoxic cells (which have a three-fold greater resistance to radiotherapy than normoxic cells) makes tumors more sensitive to the treatment.

To further understand radiosensitization, we treated separate cohorts of mice with hyperthermia, radiotherapy, both, or neither and extracted tumors 90 minutes after treatment. Just

Figure 1. Noninvasive real-time MRI shows gradual elevation of core tumor temperatures to mild hyperthermia levels.

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prior to tumor extraction, we administered markers of proliferation, perfusion, and oxygenation exogenously to all mice. Immunofluorescence staining of tumors identified fewer areas of hypoxia (and more areas of tumor proliferation) in the hyperthermia group, corroborating the DCE-MRI results.

Typically, oxygen supply depletes with distance from the feeding vessel, which leads to a hypoxic sheath of tumor cells surrounding the vascularized core of well-perfused, proliferating cells. In the combined treatment group, there was marked distortion of this structured pattern. On routine histological evaluation, this cohort had large swaths of necrosis within the tumor, while none of the others had any appreciable tissue death. We therefore hypothesized that these effects were due to vascular collapse.\(^2,3\) We confirmed the results by immunofluorescence staining for tumor microvessel density, demonstrating a substantial density decrease in the combined treatment group (see Figure 3).

The observed vascular disruption was best explained by vessel-centered focal temperature elevations. The higher temperatures were caused by perivascular sequestration of gold nanoshells too large to diffuse freely into tumor interstitium, but large enough to leak out of the vessels. We confirmed this accumulation with scanning electron microscopy. We infer that nanoshells near tumor vasculature substantially raise the focal temperature; a gradual fall-off in heat away from these areas leads to global, macroscopic tumor temperature elevations.

This non-uniformity of temperatures within tumors, with focal hot spots along the vasculature, is a unique feature of nanoshell-mediated hyperthermia. This property can be exploited for dual sensitization of tumors to radiotherapy; initially by increasing vascular perfusion of radioresistant hypoxic areas, and then by causing necrosis via vascular disruption.

Our results have shown that gold nanoshells can induce hyperthermia and may be used noninvasively. This approach enhances radiotherapy due to an acute increase in vascular perfusion and a subsequent induction of vessel collapse and necrosis.\(^3\) Our next step will establish the utility of this technique in scenarios that mimic clinical radiotherapy—where multiple small fractions of radiation are administered serially, rather than in a single large dose. We also plan to exploit the unique necrosis within tumors for other tumor-focused therapies.

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