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Anti-RhoJ antibody functionalized Au@I nanoparticles as CT-guided tumor vessel-targeting radiosensitizers in patient-derived tumor xenograft model

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ABSTRACT

The clinical success of radiotherapy is greatly hampered due to its intolerable off-target cytotoxicity induced by the high dose of radiation. Meanwhile, low dose of irradiation greatly potentiates the intratumoral angiogenesis, which promotes the local relapse and metastasis of tumor. Therefore, it is essential to reduce the irradiation dosage while inhibiting the tumor angiogenesis during radiotherapy. In this work, tumor vessel specific ultrafine Au@I nanoparticles (AIRA NPs) are fabricated and used as targeted radiosensitizers. Due to the presence of Au and iodine, these AIRA NPs exhibit superb X-ray attenuation for contrast-enhanced computed tomography (CT). Once injected, these AIRA NPs bind specifically to both newly formed tumor vessels in peri- and intratumoral regions and pre-existing tumor vessels. Upon radiation under CT guidance, AIRA NPs remarkably enhanced the killing efficacy against tumors *in vivo* with respect to radiation alone or anti-angiogenesis chemotherapy. Meanwhile, down-regulation of the level of circulating VEGF cytokine further indicates that our strategy can eradicate tumor without risking the recurrence of hypoxia and angiogenesis. Our demonstration provides a robust method of cancer therapy integrating good biocompatibility, high specificity and relapse-free manner alternative to traditional metal NPs enhanced radiotherapy.

1. Introduction

Although great achievements have been witnessed, caner remains to be the most severe threaten to the healthcare of human. Among various treatments available in clinical trials, radiotherapy (RT) is the mostly evolved one with high efficacy (>50%) [1–3]. However, its intolerable side effect and the problem of resistance developed by cancer cells during the treatment process greatly restrict its therapeutic outcome [4,5]. To address these problems, nanoparticles (NPs) comprised high atomic number elements were used as radiosensitizers to enhance the killing efficacy of radiation through potentiating the susceptibility of tumor tissue to low-dosage radiation while reducing the injury to the surrounding normal tissues [6–11]. Among all these candidates, gold NPs have been extensively studied because of their good biocompatibility, controllable size, feasible surface modification and high efficiency in generation of secondary electrons (*e.g.* Auger electrons) under irradiation to enhance radiation damage [9–11]. Furthermore, ultrafine Au NPs, exhibiting high tumor penetrating ability and surface-to-volume ratio, are proved to integrate higher sensitization efficacy with respect to larger counterparts [12]. Once accumulated in tumor, these Au NPs can change the energy distribution in the surrounding tissue upon irradiation, which selectively damages the tumor tissue while minimizing the side effect to normal tissue, thus contributing to an enhanced cancer RT [11]. On this basis, several strategies by eradicating cancerous cells in tumor lesions with metal-enhanced RT have been demonstrated [13,14].

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Although encouraging, one can imagine that the success of such strategy relies heavily on the mass accumulation of nanomedicine in tumor when considering the fact that the amount of cancerous cells is overwhelming. In this regard, cancer cells survived from RT are highly likely to achieve resistance to radiation, and progressively promote the local relapse, resulting in a poor prognosis.

Recently, a novel concept termed as targeting tumor microenvironment (*e.g.* acidic condition, up-regulated enzymes) rather than cancer cell has been proved to exhibit high therapeutic efficacy [15–18]. Encouraged by this, we questioned if similar tumor microenvironment specific RT could be realized to address these limitations of metal NPs enhanced RT. It has been widely accepted that angiogenesis plays a central role in driving tumor outgrowth and distant metastasis [19,20] Therefore, radiotherapy targeting to the tumor vasculatures would be of great benefit to suppress the tumor angiogenesis. In particular, tumor vasculatures can be divided into two categories: pre-existed vessels [21] and newly formed peri-tumoral vessels [22]. Normally, the function of pre-existed vessels is responsible for the clearance of CO_2 and metabolites [21]. The impairment of pre-existed vessels leads to significantly acidic tumor microenvironment and elevated interstitial pressure. With the accumulation of stress, hypoxic condition, characterized by extremely low partial pressure of oxygen ($pO_2 < 2.5$ mmHg), will be induced, which aggressively promotes the development of peri-tumoral vessels to alleviate hypoxia [23,24]. For the peri-tumoral vessels, they are developed mainly for the transportation of oxygen and nutrients required for the progression of cancer [22] and offer a vital avenue for distant metastasis from primary tumor [22]. Therefore, therapeutic approaches combining capabilities to eliminate the pre-existed tumor-associated-vasculature and inhibit the growth of peri-tumoral vessels simultaneously are of remarkable therapeutic importance [25]. Currently, angiogenesis inhibitors available in cancer can be mainly cataloged into two kinds: angiogenesis inhibiting agents (AIAs) and vascular-disrupting agents (VDAs) [26,27]. Particularly, AIAs are valid only for the targeting and destruction of newly developed tumor vasculatures, which means that AIAs are more suitable to inhibit the outgrowth of tumor rather than elimination [26]. Alternatively, VDAs solely target the pre-existed capillaries, leaving the newly developed vessels almost unaffected [27]. Neither AIAs nor VDAs are capable of destroy both newly formed and established tumor vascular simultaneously to realize a complete eradication of tumor.

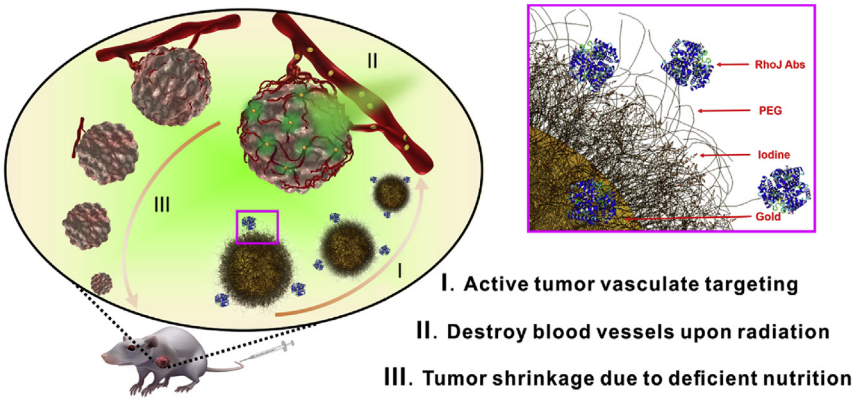
RhoJ, a member of Rho GTPase family, is receiving increasing attention in clinic due to its dominant expression on the surface of endothelial cells (ECs) of human cancer's vasculature, both of newly formed vasculatures in peri- and intratumoral regions and pre-existing tumor vessels in the intratumoral regions, while little of RhoJ is expressed in normal tissues [28,29]. Rho GTPase controls all types of angiogenic processes, including EC migration, extracellular matrix degradation, and vascular integrity, which govern the tumor angiogenesis and vascular integrity during tumor progression [30]. RhoJ blockade has proved successful to overcome the limitation of current vascular targeting systems and improve the antitumor therapy by both inhibiting tumor angiogenesis and disrupting the existed tumor vessels [31].

Thus, we envision that developing RhoJ targeting platform used in radiotherapy may merit the features from both AIAs and VDAs. In this regard, an intriguing neoplastic vasculature targeting radiosensitizer, composed of an ultrafine Au core anchored with the anti-RhoJ antibody (Ab) is developed (Scheme 1). Iodine is further conjugated to this radiosensitizer (AIRA NPs) to strengthen their X-ray attenuation ability. After *in vivo* injection of these AIRA NPs, tumor vessel networks including pre-existed deep tumor vasculature and peri-tumoral vessels can be detected under CT guidance, and selectively eliminated upon X-ray irradiation, which actively inhibits the tumor blood supply and induces the apoptosis of tumor cells in long–lasting manner after the RT. The therapeutic potential of our method is validated in Patient-Derived tumor Xenograft (PDX) models to mimic the pathological features of human cancer [32]. In this case, radiation induced apoptosis is highly localized to destroy tumor vessels without recurrence, unlike traditional low-dosage radiation, which risks relapse and metastasis induced by aggravated hypoxia [4]. This strategy would shed light on the future development of tumor microenvironment targeting RT.

2. Experimental section

2.1. Materials

Chloroauric Acid ($HAuCl_4$), ethyl dimethylaminopropylcarbodiimide (EDC) and sulfo-NHS were purchased from Sigma-Aldrich (St. Louis, MO). 5-amino-2,4,6-triiodoisophthalic acid (ATIPA), and meso-2,3-dimercaptosuccinic acid (DMSA) were purchased from Tokyo Chemical Industry Co. Ltd (Tokyo, Japan). NHS-PEG-SH (M_w~5 kDa) was purchased from Laysan Bio (Arab, AL). Dimethyl sulfoxide (DMSO) was obtained from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). All reagents were of analytical grade and used as received without further purification. α-MEM, Dulbecco's Modified Eagle Medium (DMEM), phosphate buffered saline (PBS), penicillin/streptomycin, and fetal bovine serum (FBS) were obtained from Invitrogen (Thermo Fisher Scientific, Carlsbad, CA).



Scheme 1. The illustration of AIRA NPs used as CT-guided tumor vessel-targeting radiosensitizers in radiotherapy. (These RhoJ Abs modified AIRA can target to tumor vessels and destroy them upon X-ray irradiation under CT guidance, which actively inhibits the tumor blood supply and induces the apoptosis of tumor cells in long–lasting manner after the RT); The up-right insert shows the structure of AIRA NPs.