Molecular Imaging
Volume 16: 1-4

DOI: 10.1177/1536012117730950
journals.sagepub.com/home/mix
SAGE

Rethinking Brain Cancer Therapy: Tumor Enzyme Activatable Theranostic Nanoparticles

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Abstract

This invited commentary discusses a recent article by Mohanty et al in *Molecular Cancer Therapeutics* about significant therapeutic efficacies of novel theranostic nanoparticles (TNPs) for the treatment of human brain cancers in mouse models. The TNPs were cleaved by enzymes in the tumor tissue, matrix metalloproteinase (MMP-14), which lead to release of a highly potent therapeutic drug, azademethylcolchicine. Data showed that the TNPs caused selective toxic effects in MMP-14-expressing glioblastoma and not normal brain. In addition, the iron oxide nanoparticle backbone enabled in vivo drug tracking with magnetic resonance imaging (MRI). This commentary discusses previous efforts of MMP-targeted therapeutics as well as opportunities for further refinements of tumor enzyme-activatable TNPs. If successfully translated to clinical applications, the TNPs might hold substantial potential to improving cytotoxic indexes and long-term outcomes of patients with brain cancer compared to standard therapy.

Keywords

glioblastoma, theranostics, nanoparticles, ferumoxytol, MR imaging

Our recent article by Mohanty et al in *Molecular Cancer Therapeutics*[1] reported significant therapeutic efficacies of novel theranostic nanoparticles (TNPs) for the treatment of human brain cancers in mouse models. The concept is based on the specific activation of TNPs by enzymes in the tumor tissue, matrix metalloproteinases (MMP-14), which leads to vascular damage and tumor necrosis. The TNPs consist of 3 main elements: (1) a nanoparticle backbone, (2) an MMP-14 cleavable protein linker, and (3) the potent therapeutic drug azademethylcolchicine (Figure 1).[2] After cleavage of the protein linker by MMP-14, the TNPs release azademethylcolchicine, which causes selective toxic effects in MMP-14-expressing tumor vascular endothelial cells and tumor cells. Since normal, nonneoplastic cells do usually not overexpress MMP-14, there are no significant side effect to the normal brain. In addition, the iron oxide nanoparticle backbone could be detected with magnetic resonance imaging (MRI), which enabled real-time drug tracking and could be utilized for personalized therapies.

Glioblastoma (GBM) is the most deadly primary malignant brain tumor with a median survival of less than 1 year. Although many molecular and genetic factors that drive GBM development and progression have been uncovered over the last decade, little progress has been made in the development of curative therapeutics. Despite aggressive surgical resection, radiotherapy, and chemotherapy, tumor recurrence is common and prognosis for patients with GBM remains dismal.[3-5] Glioma stem cells (GSCs) are highly resistant to chemotherapy and may be the main cause for GBM recurrence and poor outcomes.[6-8] Our data showed that azademethylcolchicine caused significant tubulin damage and apoptosis of GSC in vitro. However, in vivo, the therapeutic efficacy of azademethylcolchicine was not significantly different compared to the standard therapeutic drug temozolomide.

We postulated that the small-molecule azademethylcolchicine had unfavorable pharmacokinetics for a drug that required tumor enzyme activation in vivo: A small molecule has a short blood half-life and rapidly diffuses in and out of the tumor

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Submitted: 09/07/2017. Revised: 16/07/2017. Accepted: 18/07/2017.

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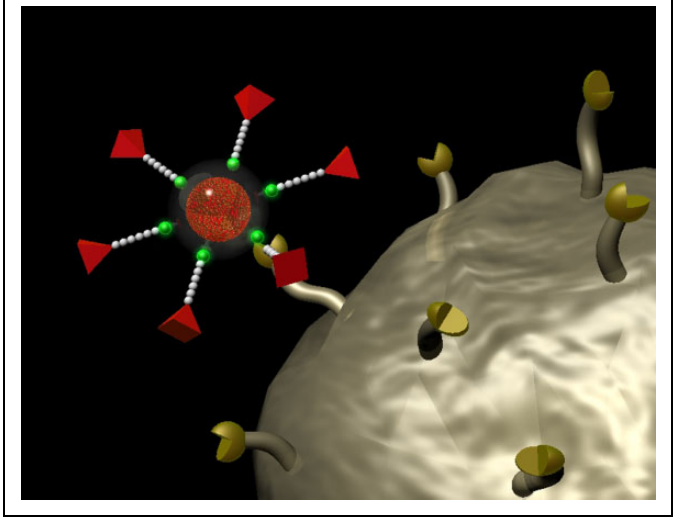

Figure 1. Cartoon of a theranostic nanoparticle (with central iron oxide nanoparticle, protein linker, and attached therapeutic drug) approaching a cancer cell.

tissue, which limits interactions with tumor enzymes. To solve this problem, we attached azademethylcolchicine to clinically applicable nanoparticles (ferumoxytol). Intravenously infused ferumoxytol nanoparticles have a blood half-life of 10 to 14 hours in humans and have been shown to accumulate in GBM in both mice and patients, where they are retained for days, presumably due to the enhanced permeability and retention effect.[9-11] Accordingly, our data showed a long blood half-life and tumor retention of azademethylcolchicine–ferumoxytol nanoparticles in GBM in mice, with significantly improved in vivo tumor toxicity compared to temozolomide alone. Since MMP-14 is both overexpressed by tumor endothelial cells and tumor cells (including glioma initiating cells), we found significant vascular damage and tumor cell necrosis in azademethylcolchicine–ferumoxytol–treated tumors, but not untreated controls. The survival time of mice with advanced orthotopic human GBM xenografts doubled after combined treatment with azademethylcolchicine–ferumoxytol plus temozolomide compared to temozolomide alone (the current clinical standard). Although untreated mice with GBM typically died after 20 to 30 days, some of the GBM-bearing mice treated with azademethylcolchicine–ferumoxytol plus temozolomide survived for more than 300 days.[1]

Matrix metalloproteinases have been studied for many decades as potential targets for tumor-specific therapy.[12] Matrix metalloproteinases are overexpressed in many cancers, including brain, breast, lung, colon, and ovarian cancers, among many others, but not normal organs. Matrix metalloproteinases degrade connective tissue between tumor cells and blood vessels and thereby enable tumor cells to escape from their original location, enter blood vessels, and seed metastases.[13] Thus, extensive efforts have been undertaken to develop drugs that can inhibit MMPs in cancers. Initial studies in mouse models had been very encouraging, and more than 50 different MMP inhibitors had been investigated in clinical trials. Unfortunately, all of these trials failed.[12] Not only was no survival benefit found, some MMP inhibitor–treated patients showed decreased survival compared to placebo-treated controls.[14] Many reasons for these disappointing results have since been elucidated, including lack of in vivo proteinase–drug interaction, lack of specificity for pro-tumoral MMPs, cross-inhibition of proteases with antitumoral effects, complex effects on tumor immune responses, among others.[12] Although improved formulations of MMP inhibitors are being explored,[15] it is important to note that our approach is fundamentally different from these previous efforts. Our TNPs do not disrupt the delicate balance of pro- and anti-inflammatory MMPs in tumors or elsewhere in the body. We do not attempt to inhibit MMP, but we rather utilize MMP-14 overexpression in cancers to achieve cancer-specific drug activation.

Since normal tissues do not usually overexpress MMP-14 and our TNP showed limited biodistribution to normal organs due to its large size, azademethylcolchicine–ferumoxytol nanoparticles could potentially provide highly selective cancer therapy with little or no side effects. Indeed, we studied the biodistribution of azademethylcolchicine–ferumoxytol in our mouse model, and our data confirmed the lack of toxicity in normal organs. No alterations were seen in histologic specimen of the brain, bone marrow, lung, heart, liver, spleen, kidneys, bowel, lymph nodes, or muscle.[16] However, since all nanoparticles and ferumoxytol specifically accumulate in the reticuloendothelial system (RES) and since liver, spleen, bone marrow, and lymph nodes can sometimes contain low levels of MMP-14, drug release in these organs could occur very slowly over time. Long-time studies will have to show whether a potential long-term low-level exposure to azademethylcolchicine in RES organs causes any significant side effects.

Apart from cancer, inflammation and wound healing can be associated with increased MMP expression.[17,18] Further studies have to show potential cross-activation of TNPs by other MMPs and resultant effects on inflammations and tissue remodeling processes. This could have important clinical implications: A patient with an inflammation or wound could experience toxic side effects at these sites after TNP treatment. Although this is already a known risk with many standard chemotherapies, toxic side effects of TNPs could be severe due to their high potency and local activation. A variety of imaging technologies have been developed for in vivo imaging of MMP expression.[19-21] Such imaging tools could become important to exclude MMP-14 overexpression in presumed normal organs prior to azademethylcolchicine–ferumoxytol therapy. In addition, the in vivo distribution of TNPs could be monitored with MRI and observations of focal nanoparticle accumulations in undesired locations could be used to adjust individualized treatment protocols.

The superparamagnetic iron oxide nanoparticle backbone of our TNPs can be used to monitor the delivery of the TNPs to cancers and specific regions within a given cancer. Following intravenous TNP infusion, a bypointense enhancement on T2-weighted magnetic resonance images indicates TNP

delivery to specific tumor areas. Our data showed that tumor areas with TNP T2 enhancement correlated with tumor areas that developed necrosis. Since the iron oxide nanoparticles are retained in the tumor tissue for several weeks, repetitive MRIs after repeated TNP administrations could be used to monitor whether TNPs are delivered to tumor areas that were previously spared. However, the TNPs do not include a biosensor for therapeutic drug activation, that is, we cannot directly visualize when, where, and how much of the therapeutic drug is activated.

The TNPs could be further refined such that the tumor-selective drug release could be imaged in real time. Currently, the differences in r1 and r2 relaxivities between the original azademethylcolchicine–ferumoxytol nanoparticles and activated products are too small to be visualized with MRI in vivo (56.0 vs 55.8 $mM^{-1}{\cdot}s^{-1}$). Future generations of TNPs could produce MRI contrast either during drug activation or as a consequence of the drugs pharmacological activity to facilitate monitoring of drug release kinetics. This may be done by attaching 2-cyano-6-aminobenzothiazole (CABT) and S-ethyl-cysteine moieties that after a loss of S-ethyl fragment and MMP peptide cleavage will cause a biocompatible condensation reaction between CABT and cysteine, which leads to aggregation of TNPs and in turn increases R_2 contrast.[22]

In summary, novel azademethylcolchicine–ferumoxytol TNPs showed encouraging results with highly effective and selective toxicity against human GBM in mouse models and little or no toxicity in the normal brain or normal organs. If further testing will yield continued positive results, the TNPs hold substantial potential to improving cytotoxic indexes and long-term outcomes of patients with brain cancer compared to standard therapy.

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: Dr Daldrup-Link, Dr Jianghong Rao (Stanford University), Dr Robert Falconer, and Dr Paul Loadman (University of Bradford) filed a joined patent application for the described theranostic nanoparticles (US14/908096 and EP20140742552).

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The reported work was in part supported by the National Cancer Institute, grants R21CA176519, R21CA190196, U54CA199075, and T32CA118681 and the University of Bradford, grant UoB-66031.

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