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A Pilot Study of Microbubble-Delivered Gene Therapy Using High Intensity Focused Ultrasound (HIFU)

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Abstract

Oncolytic adenovirus is a frequently used viral vector for gene therapy in cancer treatments. However, they are highly susceptible to liver-drainage or rejection by the immune system upon injection. This leads to an inadequate gene-delivery to the target tumor or unnecessary delivery to healthy tissues, causing necrosis in different organs. This not only decreases the effectiveness of the therapy but also creates additional damages that may lead to severe side effects, outweighing the benefit of the treatment. In order to efficiently deliver the viral vector to the target tumor and tumor tissue only, the adenovirus is inserted into microbubbles. Microbubbles have been used to locally, temporally and reversibly open the blood-brain-barrier (BBB) under the influence of high intensity-focused ultrasound (HIFU). This increases the amount of gene delivered, decreasing the dose needed to successfully induce cell apoptosis in tumor cells. Understanding that HIFU induces the opening of BBB and degradation of microbubbles at proximate sites of the target tissue, it is still unknown whether the adenovirus can efficiently and effectively be delivered to cause successful apoptosis in local tumor cells. Our study investigates the possibility of minimally-invasive gene delivery using microbubbles with MRI-guided HIFU and confirms that the adenovirus can successfully be delivered into target tumor tissues.

Introduction

One of the popular mechanisms for brain tumor treatment is gene therapy using an oncolytic viral vector targeted to tumor tissue. However, due to the blood-brain barrier (BBB) that is selective against large and therapeutic molecules, gene therapies are limited to intracranial injections [1]. This highly invasive technique can be replaced by a minimally-invasive technique in which viral vectors, like adenovirus, are injected intravenously and delivered to the brain through the bloodstream. The BBB can be opened temporally with microbubble contrast and focused ultrasound (FUS), allowing oncolytic viruses to overcome the barrier and reach target tumor tissue [2].

Ultrasound has traditionally been used as a diagnostic tool in the medical field. However, recent developments of the FUS introduced a therapeutic aspect – high frequency, high amplitude waves induce an ablative effect while low frequency, low amplitude waves induce oscillations that can be targeted to the to temporally and reversibly open the BBB [3]. These ultrasound waves are focused through a mechanism of actions similar to that of a magnifying glass [4]. Because the ultrasound waves are absorbed by the skull and are refracted, FUS incorporate thousands of transducers, leading to a more localized and penetrative delivery of sonification without the opening of the skull [3]. According to previous research, BBB opening was successfully carried out in both rabbits and humans with FUS and ultrasound contrast agents, guided by real-time MRI [5, 6].

Previous studies have shown that adenovirus itself has a limited ability in cell-to-cell spread and induction of apoptosis in tumor tissues [7]. However, the expression of relaxin gene (RLX) in adenovirus has led to an increase in even penetration and distribution of virus in tumor tissues [4]. By using relaxin-expressing adenovirus, tumor tissues can undergo apoptosis more effectively, eliminating the need to deliver higher doses of the adenovirus. However, intravenous injections are highly prone to dosage loss as most drugs are drained to the liver or unnecessarily delivered to healthy tissues [8]. This may create additional problems like liver damage or side effects that can outweigh the advantages of the treatment. Therefore, microbubbles can be used to localize adenoviruses to the target tumor area to allow lower and safer dosages while preventing unnecessary damage to healthy cells. Starting from injection of these microbubbles, real-time MRI can be used as a guiding-tool to observe adenovirus-enclosed microbubbles in the bloodstream. When these microbubbles reach the BBB, low frequency FUS can be used to open the BBB and high frequency FUS for the disintegration of microbubbles, releasing the adenovirus at the site of target tumor cells. In this study, we evaluated whether therapeutic ultrasound improves the successful delivery of GFP-tagged adenovirus into tumor cells in the brain.

Materials and methods

Cell and Animal Preparation

The human breast cancer cell line, MDA-MB-231, was grown in medium with high-glucose DMEM with 10% fetal bovine serum. A mixture of 5x104 cells in 2 µL matrigel was prepared and sterotactically injected 0.5 mm anterior and 2 mm lateral to the bregma, 3 mm deep from brain surface. Immunocompromised athymic nude mice were used in this study. After three weeks of tumor growth, tumor size was confirmed through MRI spatial coordinates of FUS positioning system co-registered to that of a 7-Tesla MRI scanner (BioSpin 7030).

Blood-Brain-Barrier Opening

After sufficient tumor growth, gadolinium (GAD) contrast agents were intravenously injected and BBB opening was verified using MR-guided focused ultrasound (MRgFUS). T1- and T2-weighted images were taken to confirm BBB opening and to detect any hemorrhage.

Oncolytic Adenovirus

GFP-tagged relaxin-expressing oncolytic adenovirus (Ad-ΔE1B-RLX) with significant viral distribution was intratumorally injected with a dosage of 8x1011-VP/kg and intravenously injected with a dosage of 1.6 x 1012 VP/kg in separate mouse models. Four mouse models were used in this study. One sample was given an intravenous injection of GFP-tagged adenovirus, delivered to normal tissue and another sample was given an intravenous injection of GFP-tagged adenovirus, delivered to tumor tissue. Another mouse model was given an intratumoral injection of GFP-tagged adenovirus and the last sample was given an intravenous injection of GFP-tagged adenovirus, delivered to tumor tissues with MRgFUS. Intravenously injected adenovirus with GFP was injected prior to MRgFUS-induced BBB opening. MRgFUS RK100 system with 1.136 MHz spherically focused transducer was used. Microbubbles were intravenously injected (20 µL/kg) before the mice was treated with FUS to create oscillations in the BBB to cause the barrier to open.

Frozen Section for GFP Confirmation

Mice were sacrificed one week after the treatment to harvest brain samples. Frozen section and fluorescence microscopy were used to confirm GFP expression of the adenovirus. Tumor cell nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI).

Results

MRgFUS induces localized and reversible BBB opening

A T1- and T2-weighted imaging of mouse model brains before adenovirus injection confirmed the opening of BBB and the absence of hemorrhage. T1-weighted MRI imaging showed a significantly greater presence of GAD in tumor tissue only after FUS treatment (Fig.1b). Tumor tissue did not display high levels expression of GAD before FUS treatment (Fig. 1a). T2-weighted MRI imaging showed a clear absence of hemorrhage after FUS treatment (Fig. 1c,d).

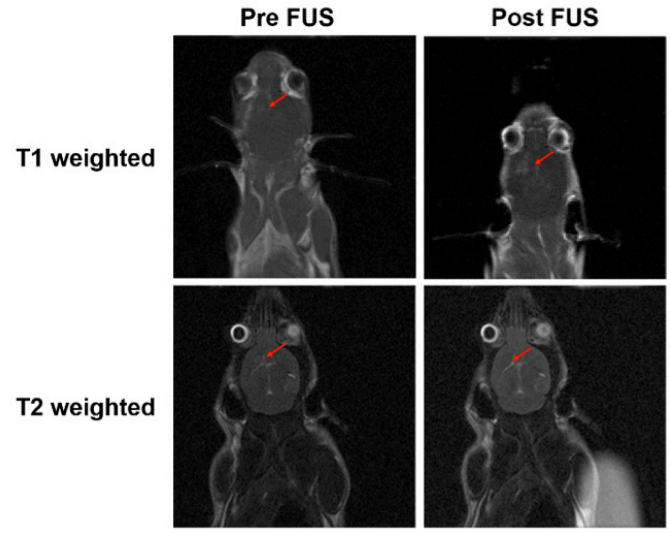

Figure 1. T1- and T2-weighted MRI of mouse model brains confirming BBB opening and absence of hemorrhage. a, T1-weighted MRI before FUS with no GAD expression and b, T1-weighted MRI after FUS with GAD expression indicating BBB opening. c, T2-weighted MRI before FUS and d, T2-weighted MRI after FUS showing no changes around tumor tissue area, indicating the absence of hemorrhage.

Intravenous or intratumoral delivery of GFP-tagged adenovirus yields poor distribution

An intravenous injection of GFP-tagged adenovirus to target normal brain tissue showed only the presence of DAPI nuclei staining and a lack of GFP expression, indicating the absence of adenovirus (Fig. 2a). Frozen section of an intravenously injected GFP-tagged adenovirus targeted to tumor tissue in the brain showed a slight increase in GFP expression, relative to normal tissue (Fig. 2b). However, adenovirus distribution was significantly uneven and the amount of adenovirus delivered was insufficient for successful gene therapy.

Intravenous delivery of GFP-tagged adenovirus using MRgFUS yields enhanced distribution and tumor penetration

Frozen section of the intratumorally injected GFP-tagged adenovirus targeted to tumor tissue confirmed the successful delivery of the virus across the tumor (Fig. 2c). Similarly, an intravenous injection of GFP-tagged adenovirus targeted to tumor tissue showed a comparably significant delivery and even distribution of the adenovirus across tumor tissue (Fig. 2d).

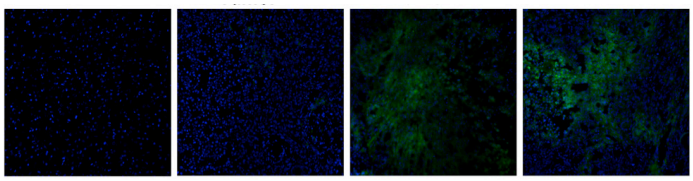

Figure 2. Frozen section of injection- and MRgFUS-dependent distribution of GFP-tagged adenovirus. a, GFP-tagged adenovirus injected intravenously to target normal tissue only displays expression of DAPI staining of nuclei. b, Intravenous injection of GFP-tagged adenovirus targeting tumor tissue in the brain has slight expression of GFP and c, Intratumoral injection of GFP-tagged adenovirus alone shows an average distribution of adenovirus across tumor tissue. d, MRgFUS-induced BBB opening prior to intravenous injection of GFP-tagged adenovirus shows similar adenovirus expression and distribution across brain tumor tissue.

Discussion

Overall, using MRgFUS significantly increased the amount of GFP expression, considering that the adenovirus was intravenously injected. This was possible due to the ability of the adenovirus to overcome the BBB, without creating novel side effects such as hemorrhage or permanent damage to the BBB. Although the intratumoral injection seems ideal and has been often the method

of gene therapy delivery to brain tumor tissue, it is considered a highly invasive treatment. In order to minimize pain and complications, treatment for tumors located deep inside the brain must be reached without opening the skull. Therefore, changing the method of delivery leads to a minimally-invasive treatment with other barriers. Because the BBB is known to keep molecules and therapeutic agents from entering the brain from the bloodstream, intravenous injection of the adenovirus, although tumor-specific, was insufficient for even distribution across tumor tissue [9, 10]. With MRgFUS that reversibly, temporally and locally opens up the BBB through oscillations in tight junctions, intravenously injected adenovirus was able to successfully reach tumor target tissue and with higher efficiency at the same dose. The dosage used for intravenous injections were much larger than the dosage used for intratumoral injections. This was to compensate for the dosage loss as the adenovirus travelled through the bloodstream. However, future experiments could further increase tumor penetration efficiency through genetic engineering and continue to lower the dosage requirement for much lower side effects and safer treatment.

Even with potent oncolytic adenovirus, there is still an uneven distribution of the virus across tumor tissue. This may be due to the different densities across the tumor or the ratio of adenovirus binding to the tumor cells. Further experiments should examine the possibility introducing microbubbles to enact oscillations in the BBB and additionally, function as another transportation vehicle for the adenovirus to reach targeted tumors. This will lead to an accurate and local release of the adenovirus and ultimately, an increase in their distribution and tumor penetration.

For this study, our goal was to overcome the BBB using MRgFUS, examining the possibility that intravenously injected relaxin-expressing adenovirus has in increased distribution in brain tumor tissues and, therefore, transform what was once a highly invasive treatment into a minimally-invasive cancer treatment. Further investigation will allow us to utilize this technique to monitor actual tumor size reduction and confirm its therapeutic efficacy by further enhancing localization and dosage compensation.

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