Magnetic nanoparticles as a new approach to improve the efficacy of gene therapy against differentiated human uterine fibroid cells and tumor-initiating stem cells

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Objective: To study whether efficient transduction and subsequent elimination of fibroid tumor-initiating stem cells during debulking of tumor cells will aid in completely eradicating the tumor as well as decreasing the likelihood of recurrence.
Design: Case control study.
Setting: Research laboratory.
Patient(s): None.
Intervention(s): Magnetic nanoparticles (MNPs) complexed to adenovirus (Ad-GFP) or (Ad-LacZ) used to transfect differentiated human fibroid cells in vitro.
Main Outcome Measure(s): Rate of transduction and tumor growth inhibition.
Result(s): We have developed a localized nonsurgical adenovirus-based alternative for the treatment of uterine fibroids that combines viral-based gene delivery with nanotechnology for more efficient targeting. Magnetic nanoparticles complexed to adenovirus, in the presence of an external magnetic field, accelerate adenovirus transduction. We observed a statistically significant increase in transduction efficiency among differentiated human fibroid cells at two different multiplicities of infection (MOI), 1 and 10, respectively, with MNPs as compared with adenovirus alone. Human fibroid stem cells transfected with Ad-LacZ expressed β-galactosidaze at a MOI of 1, 10, and 50 at 19%, 62%, and 90%, respectively, which were statistically significantly enhanced with MNPs.
Conclusion(s): When applied with adenovirus herpes simplex thymidine kinase, magnetofection statistically significantly suppressed proliferation and induced apoptosis in both cell types. Through the use of magnetofection, we will prove that a lower viral dose will effectively increase the overall safety profile of suicide gene therapy against fibroid tumors. (Fertil Steril® 2016;105:1638–48. ©2016 by American Society for Reproductive Medicine.)
Key Words: Tumor stem cells, adenovirus, cell proliferation, apoptosis

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Uterine fibroids (UFs), also known as uterine leiomyomas, are benign neoplasms of the myometrium that represent the most common solid tumor in reproductive-aged women (1, 2). These tumors occur in 77% of women overall, with a clinical manifestation in 25% of those affected by the age of 45 years (2–5). Although benign, they commonly cause severe symptoms such as heavy, irregular, and prolonged menstrual bleeding and anemia. Other common symptoms include pelvic discomfort, bowel and bladder dysfunction caused by pressure due to the anatomic placement and/or positioning of the fibroids. Uterine fibroids also have been associated with subfertility and recurrent spontaneous abortion (6–10). These clinical complications seriously impact women's health and quality of life. Uterine fibroids are the most common indication for the more than 600,000 hysterectomies performed in the United States annually. Hysterectomy, an invasive major surgery, is oftentimes associated with significant morbidity and possible mortality, and imposes a huge economic impact on the U.S. health-care delivery system (10, 11).

For women with symptomatic UFs who desire future fertility, only limited conservative methods of treatment are available to manage fibroids without compromising the subsequent chances of achieving a healthy pregnancy. Due to various factors more women are delaying childbearing, which has led to an increase in the number of nulligravida patients with symptomatic UFs (5). Despite the burden of suffering, many women affected are averse to surgery and actively seek fertility-preserving alternatives (12–14). We have previously shown that intratumoral gene therapy, a localized method of UF treatment, has the ability (15–19) to ablate UFs without interfering with ovulation, uterine blood supply, or systemic ovarian function (14, 18). The use of adenovirus complexes for the treatment of UFs serves as a novel and minimally invasive therapeutic option for this growing group of patients (14).

Adenoviruses are among the most robust gene delivery tools and offer immense promise in the field of gene therapy. Our group has a proven track record in the use of these unique vectors for the development of a localized nonsurgical alternative for the treatment of UF tumors (15–18). This technique allows for the successful ablation of UFs without interfering with ovulation, uterine blood supply, or systemic ovarian function, which are oftentimes affected by other UF treatment modalities.

Though the efficacy and safety profile of replication-incompetent adenoviruses is outstanding, their clinical use with systemic or even localized delivery is hampered by adverse reactions, including thrombocytopenia (20), acquired immune responses mediated by cytotoxic T lymphocytes against viral and/or transgene products (21, 22), and in some cases potentially life-threatening systemic cytokine syndrome (23–25). The acute toxic effects of the latter are due to activation of the innate immune system; these effects exhibit a steep dependence on vector dose—that is, a decreased viral load equates to a lessened likelihood of severe immune reaction.

Systemic cytokine syndrome occurrence varies substantially among patients, so the likelihood of eliciting such an immune response cannot be readily predicted. Our research group, has developed a method that targets therapeutic adenoviruses toward fibroid lesions and minimizes any potential delivery beyond the tumor lesion. In our approach, we have genetically modified the adenovirus with a targeting short peptide composed of three amino acids (glycine, arginine, and aspartic acid), collectively referred to as the RGD peptide motif. The RGD peptide motif is expressed on the virus capsid to use different internalization pathways other than the well-known Coxsackie-adenovirus receptor (CAR), which is commonly expressed on many normal cells (17). The advantage of the CAR-independent RGD pathway is its use of the integrin internalization pathway, which is highly expressed on fibroid tumor cells as compared with the surrounding normal myometrium (17, 26, 27).

We have found that the integration of gene therapy and nanotechnology serves as yet another approach that can be used in minimizing the required dosage of tumor-targeted adenovirus while sufficiently increasing the efficiency of transduction. When MNPs are conjugated to adenoviral vectors, in the presence of an external magnetic field they have been shown to greatly enhance the targeted gene transfer into tumor cells (28). These magnetic nanoparticles accelerate transduction kinetics, a technique referred to as magnetofection (29). The magnetofection method was developed to overcome the biological barriers against the delivery of efficient gene therapy through the use of nucleic acids or viral vectors associated with MNPs (29, 30).

The principle of magnetofection is to associate transfection reagents or viruses with specific magnetic nanoparticles, thereby forming molecular complexes. The resulting molecular complexes are then concentrated and transported into cells supported by an appropriate magnetic field (29, 30). Through the exertion of a magnetic force upon gene vectors, we were able to rapidly increase the concentration of the applied vector dose on cells so that 100% of the cells come in contact with a high vector dose, thereby promoting cellular uptake.

This approach has not yet been evaluated against human UF tumor cells. Our aim is threefold: [1] to enhance the efficiency of transduction while maintaining or minimizing viral dose, [2] to enable targeting to fibroid tumor tissue and avoid surrounding healthy myometrium, and [3] to validate that our approach can transduce and eliminate fibroid stem cell populations. The latter would be a novel paradigm-shifting improvement in UF therapeutics. Eliminating tumor-forming fibroid stem cells would likely prevent tumor recurrence, a major challenge in the field of UFs, and likely prevent the development of new fibroid lesions. Currently available treatment modalities are not capable of affecting fibroid stem cells. Use of magnetofection in fibroid gene therapy is novel and innovative, representing the natural evolution and progression of the therapeutic options available. Our group aims to pursue the design and development of cutting-edge approaches that are localized, effective, safe, and fertility preserving for the treatment of uterine fibroids. In this work, we demonstrate that magnetofection does indeed enhance adenoviral gene therapy and increase lethality against human fibroid cells. For the first time, we