Extremophile RNA delivery for Radioprotection in Prostate Cancer Patients

James D. Byrne[1,2], Ameya Kirtane[2], Netra Rajesh[2], Miguel Jimenez[2], Giovanni Traverso[2,3,4], Anthony D'Amico[5,6], Robert Langer[2,7]

Affiliations: [1] Harvard Radiation Oncology Program, Boston, MA 02114, USA. [2] David H. Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA 02142, USA. [3] Institute for Medical Engineering and Science, Massachusetts Institute of Technology, Cambridge, MA 02139, USA. [4] Division of Gastroenterology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA. [5] Department of Radiation Oncology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA. [6] Department of Radiation Oncology, Dana-Farber Cancer Institute, Boston, MA 02115, USA,4, USA. [7] Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139, USA.

Background: Prostate cancer patients undergoing radiation therapy may experience severe debilitating short- and long-term toxicities resulting in reduced quality of life and regret of their treatment decisions. These toxicities are bystander effects based on proximity of normal organs to the treatment target and may manifest as genitourinary (GU) and gastrointestinal (GI) symptoms, such as urinary frequency, urinary obstruction, and rectal bleeding. Although there have been many attempts to mitigate these toxicities using radioprotectants, there are few clinically available radioprotectants. Newer methods to reduce the incidence of GU and GI side effects may provide substantial benefit to patients.
Certain organisms in nature—known as tardigrades—have the ability to withstand extremely large doses of radiation as a result of a tardigrade-unique Dsup protein that prevents DNA damage. We propose the local delivery of mRNA for expression of the tardigrade-specific Dsup protein for radioprotection of mucosal surfaces. We hypothesize that inducing the expression of Dsup protein in normal tissues will impart a high degree of radioprotection.

Methods: A library of 200 excipients were synthesized using automation and high-throughput combinatorial chemistry. Adding the excipients to mRNA resulted in <200 nm complexes capable of cellular transfection. Screening of these excipients for transfection efficiency of a GFP mRNA was performed in human urothelial cells and evaluated using flow cytometry. The top candidates were down-selected based upon transfection efficiency, and transfection of a Dsup-Green Fluorescent Protein (GFP) mRNA was next evaluated in the human urothelial cells using these top candidates. Nuclear staining of cells with DAPI was used to confirm nuclear co-localization of the Dsup protein. After down-selection of the excipient candidates for expression of the Dsup-GFP fusion protein, the functional ability of Dsup protein to protect against DNA damage and cytotoxicity from a single dose of 10 Gy radiation were assessed using a neutral Comet assay and MTT assays, respectively.

Results: The top 10 excipients for transfection efficiency were identified among the 200 unique excipients. The top excipient candidates enabled transfection efficiencies of >60%. Nuclear localization of the Dsup-GFP protein was confirmed using a nuclear stain. In assessing DNA damage with the comet assay, cells that were pre-treated with the Dsup-GFP mRNA complexes had significantly lower amounts of DNA damage compared to their controls. The MTT cytotoxicity assay revealed significantly improved cell viability up to 8 days after pre-treatment with the Dsup-GFP mRNA complexes.

Conclusions: A combinatorial screening of excipient candidates for transfection of a Dsup-GFP protein identified the top excipient candidates. The expression of the Dsup protein in human urothelial cells significantly increased tolerance to a single dose of radiation. These agents have the potential to reduce short- and long-term radiation toxicities by reducing DNA damage.

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