mRNA-Based Delivery of Dsup Protein for Acute Radioprotection in Human Cells Without Genomic Alteration - Gamal Ahmed.pdf

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Submission date: 13-Oct-2025 11:02PM (UTC+0700)

Submission ID: 2779950057

File name: mRNA-Based_Delivery_of_Dsup_Protein_for_Acute_Radioprotection_in_Human_Cells_Without_Genomic_Alteration_-_Gamal_Ahmed.pdf (356.04K)

Word count: 1780

Character count: 10506

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Submission date: September 6, 2025

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Research topic: Molecular Biology & Biotechnology (Biomedical Application: Radioprotection)

Title: mRNA-Based Delivery of Dsup Protein for Acute Radioprotection in Human Cells Without Genomic Alteration

Abstract

Cancer remains one of the most life-threatening diseases, ranking as the second leading cause of death worldwide [1]. Radiation therapy is a crucial component of treatment for about 50% of cancer patients. However, it is often limited by severe side effects caused by radiation-induced DNA damage. Tardigrades, microscopic extremophiles, have showed remarkable resistance to radiation due to the secretion of a unique protein called damage suppressor (Dsup), which binds to DNA and reduces damage. This project explores the use of synthetic messenger RNA (mRNA) to transiently express Dsup in human cells. The aim is to enhance cellular resistance to radiation without changing the genome.

Cancer treatments:

There are several different treatments provided by healthcare, sometimes combining treatments based on the condition [2]. Common cancer treatments include:

1) **Surgery:** can remove cancerous tumors that haven't spread yet. Surgeons often use sharp tools to cut through your body and remove the cancer tumors from it [3].
2) **Chemotherapy:** destroys cancer cells with powerful drugs and prevent tumor growth. Chemotherapy is usually given intravenously (a needle through a vein) [4].
3) **Immunotherapy:** use your own immune system to fight cancer. It works by helping the immune system recognize and attack cancer cells [5].
4) **Targeted therapy:** it treats cancer by targeting specific features, changes, mutations, or substances in or on cancer cells [6].
5) **Radiation therapy:** also called radiotherapy, is a type of cancer treatment. This treatment uses beams of intense energy to kill cancer cells [7].

Radiotherapy:

Despite its effectiveness, radiotherapy has limitations. Normal tissues in the radiation field can also suffer damage, leading to side effects ranging from skin irritation and fatigue to long-term complications like fibrosis or organ dysfunction.

Side effects of radiation therapy:

The side effects of radiation depend on the part of the body that is treated, for example: the side effects of brain, are the Fatigue, Hair loss, memory or concentration problems, nausea and vomiting, the side effects of the breast are, Fatigue, hair loss, skin changes, swelling (edema), and tenderness.

As a result of these side effects scientist began to search for treatments for the side effects of radiation therapy, as radiotherapy could increase the risk of developing another type of cancer.

A type of microscopic animal called tardigrade was discovered in 1773 this animal is literally a one of a kind, an absolute hero that has remarkable abilities beyond the imagination of human beings.

Tardigrades:

They are also known as **water bears** and **moss piglets** are small, microscopic phylum Measuring less than 1 millimeter in length and has eight legs. They nearly live in all the environments, specifically, any environment with a bit of **moisture**.

Figure 1: tardigrade

Tardigrades are among the toughest animals on the planet earth, with an impressive ability to withstand extreme conditions like:

1) **Cryptobiosis:**
   a dormant state they enter when they lose almost all body water and curl into a dehydrated ball known as a "tun.". During this form they can survive years and even decades until rehydrated [9].
2) **Vacuum of outer space:**
   they are also capable of surviving the vacuum of outer space. In 2007, tardigrades were exposed to space conditions during the European Space Agency's FOTON-M3 mission. Many of the tardigrades not only survived but also reproduced after returning to Earth [10].
3) **Radiation:**
   tardigrades exhibit exceptional resistance to radiation, withstanding doses that are hundreds of times higher than the lethal limit for humans [11].

They have many other abilities like enduring the extreme pressures both the extreme high pressure and the extreme low pressure, in addition to showing resistance to toxic environments like harmful chemicals.

Tolerance to radiation:

Tardigrades have many unique mechanisms to endure radiation such as:

1) **DNA repair mechanism:**
   recent research by Anoud et al. (2024) reveals that tardigrades still accumulate DNA damage but survive by activating powerful

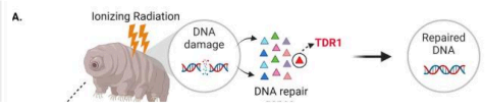

Figure 2: DNA repair mechanism of tardigrades

DNA repair mechanisms. The study identified a unique tardigrade gene encoding the protein TDR1, which binds to DNA and forms aggregates, likely helping repair fragmented DNA [12].

2) **Antioxidant Defense Systems**
3) **Cryptobiosis and Water Removal**

Damage Suppressor Protein (Dsup)

Ramazzottius varieornatus and Hypsibius exemplaris are two species of tardigrades that produce a unique protein called Damage Suppressor Protein (Dsup). This protein gives them the ability to tolerate radiation doses **2000 to 3000** times more than humans.

Dsup is a highly charged and largely unstructured nuclear protein that binds to DNA. DNA is intricately packaged to fit inside the nucleus while allowing controlled access for cellular functions. The packaging begins with DNA wrapping around histone proteins, forming structures called nucleosomes, which resemble "beads on a string." Each nucleosome consists of about 147 base pairs of DNA wound around a histone octamer. These nucleosomes coil into a more compact structure called chromatin. Chromatin further folds into loops and is anchored to a protein scaffold, forming higher-order structures. During cell division, chromatin condenses even further to form visible chromosomes.

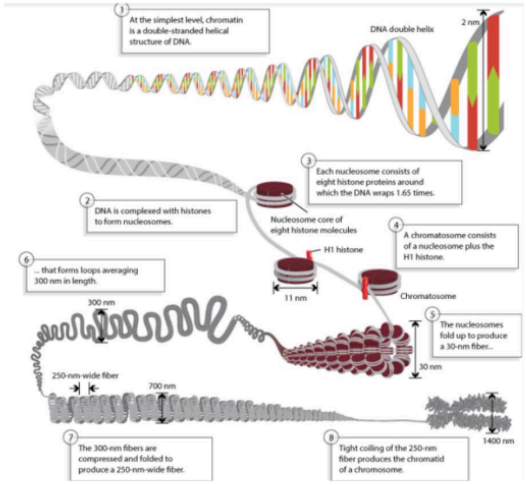

Figure 3: DNA packaging

Dsup plays a critical role in protecting DNA from damage caused by **ionizing radiation** and **reactive oxygen species (ROS)**, such as hydroxyl radicals. This **445-amino-acid** protein is intrinsically disordered, meaning it lacks a fixed structure, allowing it to adopt flexible conformations that enable tight binding to DNA and nucleosomes, the packaged form of DNA in eukaryotic cells. Dsup's protective mechanism relies on its **high positive charge**, with **59 lysines** and **12 arginines**, facilitating strong **electrostatic interactions** with the negatively charged phosphate backbone of DNA. Computational studies and molecular dynamics simulations reveal that Dsup forms a "fuzzy complex" with DNA, maintaining its disordered state even when bound, which allows it to shield DNA from damage without significant structural changes. This flexibility enables Dsup to provide a protective "electric shield," reducing DNA strand breaks caused by radiation or oxidative stress. Dsup's **nucleosome-binding domain** shares similarity with **vertebrate HMGN proteins,** suggesting a conserved mechanism for chromatin interaction.

Studies show that Dsup binds more tightly to **nucleosomes** than free DNA, protecting chromatin from hydroxyl radical-mediated cleavage [13].

Experimental Trials and Methodology:

In Vitro and In Vivo Data:

In human HEK293 cells, Dsup expression via mRNA reduced X-ray-induced DNA damage by ~40–50%. In MIT's 2025 mouse trials, mRNA encoding Dsup was injected, generating protein levels sufficient to protect cells. Exposed to radiation (e.g., 2–7 Gy), Dsup-expressing mice showed 50% fewer DNA strand breaks in tissues like colon and mouth, with biodistribution confirming targeted delivery (transfection efficiency ~70%, protein expression peaking at 24–48 hours post-injection) [14].

Nanoparticle Library Details:

To optimize delivery, libraries of hybrid lipid-polymer nanoparticles were screened. Components included ionizable lipids (e.g., ALC-0315 for mRNA binding), cholesterol (for stability), PEGylated lipids (e.g., DMG-PEG for stealth), and helper lipids (e.g., DSPC for uptake), combined with polymers like polyethyleneimine (PEI) or poly(lactic-co-glycolic acid) (PLGA) for controlled release. Generated via microfluidics, varying ratios (e.g., 40:10:48:2 lipid:cholesterol:polymer:PEG), surface modifications, and ligands (e.g., for colon-targeting via lectins). High-throughput in vitro screening on HEK293 cells evaluated transfection (>80% efficiency), mRNA stability (>90% intact after 24h), endosomal escape, and cytotoxicity (<10% cell death). In vivo validation in mice assessed pharmacokinetics (half-life ~12h), biodistribution (e.g., 60% in colon), and efficacy under 5 Gy radiation. Machine learning (e.g., random forest models) predicted optimal formulations based on 1,000+ variants.

Comparison to Existing Radioprotective Agents:

Unlike amifostine, a thiophosphate cytoprotectant that scavenges free radicals but causes side effects like hypotension, nausea, and requires IV administration, Dsup offers targeted, transient protection without systemic toxicity. Amifostine reduces xerostomia in head/neck cancer (51% vs. 78% grade ≥2 with placebo) but is less effective at high doses (>7 Gy) and non-specific. Dsup, via mRNA, provides DNA-specific shielding, reducing damage by 40–50% in cells, with potential for localized delivery, making it superior for radiotherapy.

Computational Approaches:

Computational modeling enhanced design: Molecular dynamics simulations (e.g., using GROMACS) modeled Dsup-DNA interactions, confirming binding affinity (Kd ~10 nM) and stability under radiation. Nanoparticle screening used AI-driven models (e.g., neural networks) to predict lipid-polymer compatibility and cellular uptake. Bioinformatics tools like AlphaFold predicted Dsup's disordered structure, while docking simulations (AutoDock) optimized nanoparticle-mRNA binding.

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