Toxic Effects of Increasing Concentrations and Treatment Durations: Nuclear-Contaminated Water Kills Triple-Negative Mammary Carcinoma Cells by Means of Elevated ROS and DNA Damage

Han Meng

Department of Du Yan, Capital Normal University, Beijing, China
1220604094@cnu.edu.cn

Abstract: Nuclear-contaminated water, a concerning byproduct of nuclear mishaps and industrial operations, contains radioactive elements. These substances pose substantial threats to both human health and the environment. Since the release of processed water contaminated with nuclear substances into the Pacific Ocean in 2023, global concerns have been mounting. In this investigation, an analysis is conducted on the cytotoxic and genotoxic effects that nuclear-contaminated water exerts on MDA-MB-231 cells. MDA-MB-231 is a cell line typical of triple - negative breast cancer (TNBC), which is recognized for its aggressiveness and poor prognosis. We suspect that nuclear-contaminated water causes MDA-MB-231 cells to enter a death pathway by ramping up reactive oxygen species (ROS) production and inflicting DNA damage. With the utilization of MTT assays, flow cytometry (CellROX), TUNEL assays, and Western blot analysis, we assessed cell viability, ROS production, breaks in DNA double-strands, as well as the existence of DNA damage-related proteins like PARP and p53. Based on how we designed the experiment, we think nuclear-contaminated water will raise ROS levels significantly, lower cell viability, and inflict DNA harm on MDA-MB-231 cells, thereby backing up our hypothesis. These findings show that nuclear-contaminated water has certain potential health risks and help us understand how it's toxic, especially in relation to breast cancer. Our study really highlights the need for more research on the environmental and biological effects of radioactive contaminants.

Keywords: Nuclear-contaminated water, Triple-negative breast cancer, Reactive oxygen species, Cytotoxic, Double-Strand Break

1. Introduction

Nuclear-contaminated water, generated from nuclear accidents, cooling procedures in nuclear plants, and nuclear explosions, is filled with high levels of radioactive substances. The Fukushima Daiichi nuclear disaster in 2011, set off by an earthquake registering magnitude 9.0 and the ensuing tsunami, led to the meltdown of three reactors and the accumulation of a substantial quantity of polluted water. Even after treatment with the Advanced Liquid Processing System (ALPS), this water still harbors radioactive elements such as tritium, cesium-137, and strontium-90. These elements are extremely dangerous to marine ecosystems along with human well-being [1]. On 24th August 2023, Japan began

discharging this so-called processed water into the Pacific, sparking global controversy [2] and raising concerns about its long-term environmental and health impacts [3].

Human bodies are quite vulnerable to radioactive substances, which can get into our system through eating, breathing, or even through our skin and then spread to different tissues [4], including breast tissue. These substances emit high-energy particles capable of directly damaging cellular DNA, leading to mutations, chromosomal aberrations, and genomic instability [5]. Additionally, they also cause an excessive buildup of reactive agents, which throws off the balance of oxidation and reduction inside the cell and causes oxidative stress. This oxidative stress can speed up the process of cells becoming cancerous [6]. All these mechanisms show how dangerous it can be to be exposed to nuclear-contaminated water, especially in terms of cancer risk.

Breast cancer, especially triple-negative breast cancer (TNBC), is still a major problem for global health. TNBC is different because it doesn't have the estrogen receptor (ER), progesterone receptor (PR) and the absence of the second type of human epidermal growth factor receptor (HER2), resulting in poor differentiation, high invasiveness [7], and a dismal five-year survival rate beneath 15% [8]. The cell line MDA-MB-231, which is often used to study TNBC, shows very invasive and metastatic behavior, just like what we see in real patients [9]. Because it doesn't have those receptors, it's a great model for studying how environmental things that can cause cancer, like radioactive substances, work. For example, when we expose these cells to nuclear-contaminated water, any changes we see in how the cells act are more likely to be directly because of the radiation damage, not mixed up with signals from receptors [10].

Radioactive elements in nuclear-contaminated water, like tritium, cesium-137, and strontium-90, release high-energy particles that can damage DNA and mess up how cells work [11]. In breast cancer research, these effects are really important because they can have a big impact on how tumors start and grow. With the number of breast cancer cases going up around the world and not many good treatment options for TNBC, it's really important to understand how environmental factors, like nuclear-contaminated water, may potentially contribute to the causation of cancer.

Aim to figure out how nuclear-contaminated water affects MDA-MB-231 cells in terms of cell death and DNA damage. By looking at how the cells multiply, die (apoptosis), the condition of their DNA, and how much oxidative stress there is, we hope to learn more about the health risks from radioactive contaminants and whether they might be involved in breast cancer development. Our results will not only help us understand better how nuclear-contaminated water affects biology but also add to the big discussion about what in the environment can cause cancer and how to prevent it.

2. Hypothesis

Predict that increasing concentrations and treatment durations Nuclear-contaminated water kills triple - negative mammary carcinoma cells by means of elevated ROS in combination with DNA damage.

3. Materials and methods

Test ROS by CellRox flow cytometry (FACS) assay, DNA fragmentation by TUNEL assay using EdUTP, cell viability by MTT assay. Moreover, the cleavage of poly (ADP-ribose) polymerase (PARP) is identified via western blot analysis. Paclitaxel serves as the positive control, while the negative control is phosphate-buffered saline (PBS).

3.1. Cell culture and treatment

MDA-MB-231 cells are cultivated in Dulbecco's Modified Eagle Medium (DMEM), which is supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. These cells are incubated at 37°C within a humidified environment containing 5% CO_2. For treatment, cells are

seeded in appropriate culture plates and permitted to attach overnight. Then, these cells are exposed to nuclear-contaminated water samples at different concentrations. These concentrations are obtained by diluting the initial stock solution, which is prepared based on actual nuclear-contaminated water, to 1 Bq/L (a 1000-fold dilution), 10 Bq/L (a 100-fold dilution), and 100 Bq/L (a 10-fold dilution). Phosphate-buffered saline (PBS) serves as the negative control. Besides, paclitaxel is utilized as the positive reference at concentrations of micromolar (μM), 5 micromolar (μM), 10 micromolar (μM), and 20 micromolar (μM). Each treatment is carried out in triplicate and the treatment durations are set at time - points of 24, 48, and 72 hours.

3.2. CellRox flow cytometry (FACS) assay

A flow cytometer is used for analyzing the fluorescence-labeled cells and is calibrated prior to each experiment. Centrifuges are utilized to harvest and wash the cells, and incubators with a temperature-controlled environment of 37°C and a 5% CO_2 atmosphere are employed to culture the cells. The CellRox reagent is purchased from an appropriate supplier and stored at a suitable storage temperature in the dark. An applicable cell depository provides the MDA-MB-231 cells.

Subsequent to exposure to varying concentrations of nuclear-contaminated water samples and the positive and negative controls for the specified time spans of 24-hour, 48-hour, and 72-hour periods, the growth medium is decanted, and then the cellular specimens are subjected to a double - rinse with phosphate-buffered saline (PBS). Next, 1 mL of the prepared CellRox solution (at a concentration of 5 μM) is pipetted into every well. The cell samples are then a temperature of 37°C for 30 minutes within a light-free environment. Once the incubation is completed, those cells are harvested by trypsinization, subjected to centrifugation at 1000 revolutions per minute (rpm) for 5 minutes. After centrifugation, the supernatant liquid is carefully decanted. Subsequently, the cells are re-dispersed in 500 microliters of phosphate-buffered solution (PBS). Right after that, the mixture of suspended cells is analyzed using the flow cytometer. The data acquisition is set to collect at least 10,000 events per sample. The fluorescence intensity of CellRox-labeled cells is measured, and the ROS levels are determined based on the fluorescence intensity. The comparison groups would be the cells treated with different concentrations of nuclear-contaminated water samples at different time points compared to the positive control (paclitaxel-treated cells) and the negative control (PBS-treated cells) to assess the impact of the nuclear-contaminated water on ROS production relative to the controls.

3.3. TUNEL assay

A fluorescence microscope is used to observe the fluorescence-labeled cells, and a microplate reader is available for quantitative analysis if necessary. A humidity-controlled chamber is used during the TUNEL reaction.

EdUTP is sourced from an appropriate supplier, Terminal deoxynucleotidyl transferase (TdT) enzyme is sourced from an appropriate supplier, and the TUNEL reaction buffer is formulated in accordance with the manufacturer's protocol. Fixative: 4% paraformaldehyde is purchased from an appropriate supplier and dissolved in PBS. The solution for permeabilization is composed of 0.1% Triton X-100 in PBS.

After treatment as described above for the set concentrations and durations, the cells are fixed using 4% paraformaldehyde for a duration of 15 minutes at room temperature. Then, the permeabilization of the fixed cells is carried out with 0.1% Triton X-100 in PBS for 10 minutes. The TUNEL reaction mixture is prepared by adding EdUTP and TdT enzyme to the reaction buffer and put into the permeabilized cells. Place the cells in a humidity-controlled chamber. Let them incubate at 37°C for 60 minutes while keeping the environment dark. After the incubation period has ended, wash the cells three times using PBS. Proceed to observe the fluorescence-labeled cells through a

fluorescence microscope. If quantitative analysis is required, the cells are trypsinized, harvested, and resuspended in PBS and moved to a 96-well plate for analysis using a microplate reader. The comparison groups are the cell populations exposed to varying concentrations of nuclear-contaminated water specimens at different time points compared to the positive control and the negative control to evaluate the effect of the nuclear - contaminated water on DNA fragmentation relative to the controls.

3.4. MTT assay

An absorbance microplate reader is employed to determine the absorbance values of the formazan solution, and incubators are used for maintaining the cell culture.

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) is sourced from an appropriate supplier and solubilized in PBS to a stock concentration of 5-mg/mL and then filtered using a 0.22-µm filter. Dimethyl sulfoxide (DMSO) is from an appropriate supplier.

After treating the cells with the various concentrations of nuclear-contaminated water specimens, and setting up positive and negative controls for 24, 48, and 72 hours, every well within the 96-well microplate receives 100 µL of a 0.5-mg/mL MTT solution. Then, the microplate is subjected to a 4-hour incubation period at 37°C. Upon completion of the incubation phase, the culture medium is meticulously decanted, and 150 µL of dimethyl sulfoxide (DMSO) is added to each well to dissolve the formazan crystals. Subsequently, the microtiter tray is agitated gently for a duration of 10 minutes at ambient temperature. Use a microplate reader to measure the absorbance of each well's solution at 570-nm wavelength. The cell viability is worked out based on the absorbance figures and compared among different treatment groups. The comparison groups are the cell populations exposed to varying concentrations of nuclear-contaminated water specimens at different time points compared to the positive control and the negative control to assess how nuclear-contaminated water impacts cell viability, contrasting the results against control samples.

3.5. Western blot

A vertical electrophoresis system, a semi-dry transfer apparatus, an orbital shaker, and a chemiluminescence imager were used.

Anti-PARP primary antibody was purchased from an appropriate supplier, and the secondary antibody that has been covalently coupled to horseradish peroxidase (HRP) was from an appropriate supplier. Electrophoretic separation reagents including anionic surfactant (sodium lauryl sulfate), acrylamide monomer crosslinker system (acrylamide/bis-acrylamide co-polymerization components), radical polymerization initiators (TEMED and ammonium persulfate catalytic pair) were utilized. Additional essential materials comprised protein transfer medium, signal suppression solution formulated as 5% (w/v) defatted milk solids in Tween-20 modified Tris-saline buffer (TBST), cellular homogenization solution incorporating protease inactivation cocktails, along with bicinchoninic acid-based protein quantification system. All chemical regents and analytical kits were procured from certified biochemical suppliers.

After treating the cells with set concentrations of nuclear-contaminated water specimens and establishing positive and negative controls, cells are initially washed twice with ice-cold PBS for durations of 24, 48, and 72 hours respectively. Next, an appropriate quantity of lysis buffer is added to each well, followed by incubating the cells on ice for 15-30 minutes. Subsequently, the cell lysate undergoes centrifugation at 12,000 revolutions per minute (rpm) for 15 minutes at 4°C. Then, the supernatant containing total protein is transferred to a new tube. The protein concentration within the cell lysate is ascertained using the BCA protein assay kit. Thereafter, take equivalent amounts of protein and mix them with an equal volume of 2× sample loading buffer. After that, heat the mixture

at 95-100°C for 5 minutes. Finally, load the sample onto a 10% polyacrylamide gel and run a constant-voltage electrophoresis at 120 V until the bromophenol blue dye reaches the gel's bottom.

Following electrophoretic separation,polypeptides are electrophoretically transferred onto nitrocellulose substrate using horizontal electroblotting equipment. The immobilized proteins are initially passivated through immersion in TBST (Tris-HCl buffered saline containing 0.1% polysorbate 20) supplemented with 5% (w/v) skim milk powder for 90-120 minutes under standard laboratory conditions. Subsequent immunodetection involves extended cold exposure (12-16 hr at 4°C) to species-specific immunoglobulin targeting PARP epitopes,prepared at 0.1% concentration ratio in identical blocking buffer. Three sequential buffer exchanges (5-min intervals) using fresh TBST precede application of enzyme-linked detection reagent-specifically,peroxidase-conjugated species-matched IgG diluted 5000-fold in milk-containing TBST-with room temperature incubation lasting 60-120 minutes. Post-incubation,the matrix undergoes triple TBST rinsing before chemiluminescent signal generation using commercial peroxidase substrate solution. Digital image acquisition captures light emission patterns corresponding to antigen-antibody complexes. Bands corresponding to PARP and cleaved PARP are visualized with the chemiluminescence imager, and relative levels of PARP cleavage are analyzed.

Comparison groups consist of cell populations exposed to varying concentrations of nuclear-contaminated water specimens at different time points, compared with positive and negative controls, to examine the impact of nuclear-contaminated water on PARP cleavage relative to the controls.

3.6. Statistical analysis

Every single experiment is performed in triplicate. All data obtained from the flow cytometry, fluorescence microscopy, microplate reader, and western blot are analyzed using statistical software (e.g., GraphPad Prism). Parametric comparison of bivariate central tendency was conducted through implementation of the independent samples t-test procedure. For multigroup mean evaluations, single-factor variance decomposition (ANOVA) was systematically applied. Upon detection of significant omnibus effects ($\alpha=0.05$ threshold), comprehensive pairwise contrast evaluations employing the Tukey HSD method were executed to delineate specific intergroup differentials.

4. Results

According to Table 1, we predicted the impacts of nuclear-contaminated water on MDA-MB-231 cells. It needed to employ multiple detection methods, including MTT, CellROX FACS assay, TUNEL assay, and Western Blot. The following table presents the combination of possible results (CR) and the corresponding findings, along with the support level for the hypothesis.

Table 1: The effects of nuclear - contaminated water on MDA - MB - 231 cells

Combination of possible results (CR)	Nuclear-contaminated water kills MDA - MB - 231 by MTT	Nuclear-contaminated water increases ROS by CellROX FACS assay	Nuclear-contaminated water increases DNA DSB by TUNEL assay	Nuclear-contaminated water increase poly (ADP - ribose) polymerase (PARP) cleavage by Western Blot	Support hypothesis
CR1	+	+	+	+	Fully yes
CR2	+	+	+	-	Partial

Table 1: (continued)

CR3	+	+	-	+	Partial
CR4	+	-	+	+	Partial
CR5	-	+	+	+	Partial
CR6	+	+	-	-	Partial
CR7	+	-	-	+	Partial
CR8	-	-	+	+	Partial
CR9	+	-	+	-	Partial
CR10	-	+	-	+	Partial
CR11	-	+	+	-	Partial
CR12	+	-	-	-	Partial
CR13	-	+	-	-	Partial
CR14	-	-	+	-	Partial
CR15	-	-	-	+	Partial
CR16	-	-	-	-	Fully Contradicts

Table legend:

"+" indicates that the measured parameter shows an increase or activation trend compared to the positive control (paclitaxel-treated group) or in comparison with the negative control (PBS-treated group) as expected by the hypothesis;

"-" indicates that the measured parameter shows a decrease or inhibition trend compared to the positive control or in comparison with the negative control contrary to the expected hypothesis.

Combination of possible results 1(CR1):

If I obtain this result, CellRox flow cytometry would identify an obvious upsurge in ROS levels of MDA-MB-231 cells. The TUNEL assay or γ-H2AX immunofluorescence staining would show increased DNA fragmentation. In the MTT assay, the cell viability would be relatively low. And Western Blot analysis would reveal that the manifestation of DNA damage-related proteins (such as PARP and p53) is upregulated compared to the control group. These results fully support the view that nuclear - contaminated water has a significant cytotoxic effect on MDA-MB-231 cells.

Combination of possible results 2(CR2):

If I obtain this result, the increase in ROS levels, DNA fragmentation, and decreased cell viability would be in line with the hypothesis. However, the Western Blot result showing no upregulation of DNA damage response-related proteins would suggest that there might be other factors influencing this aspect or that the nuclear-contaminated water's effect on this pathway is not as expected, indicating partial support for the hypothesis.

Combination of possible results 3(CR3):

If I obtain this result, I would find an increase in ROS levels, PARP cleavage, and decreased cell viability. Yet, contrary to expectations, there are no oligonucleosomal cleavage events, which indicates partial support for the hypothesis.

Combination of possible results 4(CR4):

If I obtain this result, I would observe a lack of increase in ROS levels but the presence of DNA fragmentation, decreased cell viability, and PARP cleavage, which would indicate partial support for the hypothesis.

Combination of possible results 5(CR5):

If I obtain this result, I would notice an increase in ROS levels and DNA fragmentation, but the cell viability not decreasing as hypothesized and the presence of PARP cleavage, which would indicate partial support for the hypothesis.

Combination of possible results 6(CR6):

If I obtain this result, I would observe an elevation in ROS levels concomitant with diminution of cell viability, but no DNA fragmentation and no PARP cleavage, which would indicate partial support for the hypothesis.

Combination of possible results 7(CR7):

If I obtain this result, I would find a lack of increase in ROS levels and DNA fragmentation but a decrease in cell viability and PARP cleavage, which would indicate partial support for the hypothesis.

Combination of possible results 8(CR8):

If I obtain this result, I would see a lack of increase in ROS levels and a decrease in cell viability but the presence of DNA fragmentation and PARP cleavage, which would indicate partial support for the hypothesis.

Combination of possible results 9(CR9):

If I obtain this result, I would notice a lack of increase in ROS levels and PARP cleavage but the presence of oligonucleosomal cleavage events and attenuation of metabolic activity retention, which would indicate partial support for the hypothesis.

Combination of possible results 10(CR10):

If I obtain this result, I would notice an increase in ROS levels and PARP cleavage, with no DNA fragmentation and no decrease in cell viability, which would indicate partial support for the hypothesis.

Combination of possible results 11(CR11):

If I obtain this result, I would see an increase in ROS levels and DNA fragmentation, but the cell viability not changing as expected and no PARP cleavage, which would indicate partial support for the hypothesis.

Combination of possible results 12(CR12):

If I obtain this result, I would observe a lack of increase in ROS levels, DNA fragmentation, and PARP cleavage, but decreased cell viability, which would indicate partial support for the hypothesis.

Combination of possible results 13(CR13):

If I obtain this result, I would find an increase in ROS levels only, while the other parameters show no change as expected. This would indicate that there might be strong cellular defense mechanisms against the nuclear-contaminated water's effects on DNA, cell viability, and PARP cleavage or that the measurements are affected by other factors, providing only partial support.

Combination of possible results 14(CR14):

If I obtain this result, I would observe a lack of increase in ROS levels and PARP cleavage and no change in cell viability but the presence of DNA fragmentation, which would indicate partial support for the hypothesis.

Combination of possible results 15(CR15):

If I obtain this result, I would find a lack of increase in ROS levels, DNA fragmentation, and cell viability but the presence of PARP cleavage, which would indicate partial support for the hypothesis.

Combination of possible results 16(CR16):

If I obtain this result, I would see no notable differences in all tested indicators versus control group. This would completely contradict the hypothesis that nuclear-contaminated water impacts breast cancer cell.

5. Possible results for the variables of concentration and treatment duration

The expected experimental results of flow cytometry indicate that with increasing concentrations of diluted nuclear-contaminated water (10×, 100×, 1000×) and prolonged treatment durations (24h, 48h, 72h), breast cancer cells exposed to treatment showed higher levels of stress-related molecules than untreated cells, indicating increased oxidative activity. The TUNEL assay is expected to show that

under escalating concentrations and extended durations of nuclear-contaminated water exposure, the DNA fragmentation degree in triple-negative breast cancer cells is expected to rise. The MTT assay is projected to demonstrate a gradual decline in MDA-MB-231 cell viability with higher concentrations and longer treatment times of nuclear-contaminated water. Western blot analysis is anticipated to reveal upregulated expression of proteins such as p53 and PARP within triple-negative breast cancer models following dose- and time-dependent treatment with diluted nuclear-contaminated water.

Collectively, these simulated results directly support the hypothesis that nuclear-contaminated water induces oxidative stress, DNA damage, and cytotoxicity in a concentration-dependent and time-dependent manner.

6. Discussion

This study is designed to investigate how radioactive water exposure impacts MDA-MB-231 triple-negative breast cancer cells by systematically evaluating changes in cell survival, oxidative stress levels (ROS), DNA integrity, and apoptosis markers (PARP cleavage) across varying concentrations and treatment durations.

7. Analysis of possible results

Cell Viability: For cell viability, it is expected that as the concentration of nuclear-contaminated water increases and the treatment time prolongs, the cell viability will gradually decrease. High concentrations of nuclear-contaminated water may directly damage the metabolic function of cells, disrupt the integrity of the cell membrane, or interfere with intracellular signaling pathways, thereby inhibiting cell growth and proliferation [12]. In a short time, cells may maintain a certain viability through their own repair mechanisms [13]. However, as the treatment time continues, the damage accumulates, and the cell viability eventually decreases. For example, after 24 hours of treatment, low concentrations of nuclear-contaminated water (such as 1000x) may have a small impact on cell viability. However, following 72 hours of treatment, even at low concentrations may cause a significant decrease in cell viability, while high concentrations (such as 10x) may lead to a sharp decline in cell viability in a shorter time.

ROS Production: As the concentration of nuclear-contaminated water increases, it is expected that the ROS levels in MDA-MB-231 cells may increase correspondingly. Higher concentrations of nuclear-contaminated water may introduce more reactive components or trigger stronger cellular stress responses, leading to an imbalance in the intracellular redox state and the generation of more reactive oxygen species [14]. At lower concentrations, cells may maintain a relatively stable ROS level through their own antioxidant defense mechanisms. However, when the concentration exceeds a certain threshold, the antioxidant system may be overwhelmed, resulting in a large accumulation of ROS [15]. For example, at highly diluted levels of radiologically tainted water (e.g., 1:1000 ratio), the ROS level may gradually increase. Under strongly concentrated radioactive water conditions (e.g., 1:10 ratio), the increasing trend may be more significant.

Degree of DNA fragmentation: Regarding DNA fragmentation, at increased radioactive contaminant loads, cellular systems exhibit diminished capacity to maintain DNA topological stability, resulting in an increase in the degree of DNA fragmentation. In terms of treatment time, as the time prolongs, DNA damage may accumulate gradually, evolving from early minor damage to obvious fragmentation [16]. After 24 hours of treatment, only a small amount of DNA breakage may occur, but after 48 hours and 72 hours, the degree of fragmentation may increase significantly, and the change in the high-concentration treatment group is more obvious.

DNA Damage Response Protein Expression: Concerning the expression of proteins associated with the DNA damage response, as the concentration of water contaminated by nuclear substances rises and the treatment time prolongs, it is expected that the expression of proteins such as p53 and PARP will be upregulated. This is because the nuclear-contaminated water may cause nucleic acid structural lesions, inducing phosphorylation cascades in the DDR signaling network [17]. At lower concentrations and shorter treatment times, the upregulation may be relatively mild. But as the concentration and time increase, the expression of these proteins may increase significantly, indicating a more severe DNA lesion recognition and repair coordination.

Mechanistic Role of p53 in Hypothetical Pathways: As a master regulator of cellular stress responses, p53 acts as a crucial central node that integrates DNA damage and oxidative stress signals [18] induced by nuclear-contaminated water. Once exposed to nuclear-contaminated water, radionuclides can generate reactive oxygen species (ROS) through radiolysis [19]. Resulting DNA damage includes both 8-oxoguanine modifications and DSBs, where DSB sites become phosphorylated at H2AX serine 139 [20]. These DNA insults activate ATM kinase, which in turn phosphorylates p53 at Ser15. This phosphorylation event stabilizes the p53 protein and enhances its transcriptional activity [21]. Activated p53 then upregulates the expression of CDKN1A (p21), inducing a checkpoint-mediated blockade of G1 to S transition. This arrest facilitates DNA repair via pathways like base excision repair (BER) [22]. However, when the DNA damage persists, p53 -dependent apoptosis is triggered. This occurs through upregulating apoptosis-inducing transcripts including BAX and PUMA. These genetic alterations induce mitochondrial permeability transition, resulting in cytochrome c release and subsequent activation of apoptotic caspase-3-mediated PARP cleavage [23]. This apoptotic cascade is consistent with the hypothesis of upregulated expression levels of p53 and PARP proteins observed in the Western blot experiment.

Research Significance of ROS and DNA Damage: ROS, generated as a consequence of radionuclides in nuclear-contaminated water, initiate a series of events culminating in DNA damage [24]. At the cellular level, such damage disrupts normal cell functions. It can trigger cell cycle arrest as a protective mechanism or, in more severe cases, lead to apoptosis [20]. Persistent or irreparable DNA damage often results in mutations. These mutations are a key driver in transforming normal cells into cancerous cells, thereby initiating the carcinogenic process [25]. For existing cancer cells, DNA damage further exacerbates their aggression, enhancing metastatic potential and treatment resistance.

In ecological systems, organisms exposed to nuclear - contaminated water face significant threats. Aquatic species, for instance, may experience impaired reproduction, growth, and development due to ROS-induced DNA damage [26]. This has the potential to disrupt entire food chains, leading to ecological imbalances. From a human health perspective, long-term exposure to nuclear-contaminated water, whether through ingestion, inhalation, or dermal contact, substantially increases the risk of developing various diseases. These consequences can be far-reaching, manifesting as conditions like cancer, genetic anomalies, and immune system glitches [27][28].

Basically, figuring out how nuclear-contaminated water leads to the production of ROS and subsequent DNA damage is super important. This knowledge is key for accurately sizing up and effectively lessening the long-term risks tied to nuclear pollution. It's a crucial part of keeping our environment in good shape and protecting people's health.

8. Conclusion

In this study investigating how nuclear-contaminated water impacts MDA-MB-231 breast cancer cells, we explored several possible results.

The findings show that nuclear-contaminated water exhibits a complex interaction between nuclear-contaminated water and MDA-MB-231 cells. When the concentration of nuclear-

contaminated water goes up and the treatment time gets longer, it's very likely that cell viability will drop, which means the water is toxic to the cells. Along with this, there might be more ROS produced, which can cause oxidative stress [29]. Additionally, the presence of DNA damage, as detected by methods such as the TUNEL assay and Western blot assay, implies the activation of DNA damage response pathways [30]. These results strongly suggest that nuclear-contaminated water can cause both cell-killing and DNA-damaging effects on MDA-MB-231 cells by increasing ROS levels and damaging DNA.

The observed DNA damage, characterized by increased DSBs (detected by TUNEL assay) concomitant with DNA repair protein accumulation, is of particular concern [31]. It is likely that the alterations in DNA structure caused by nuclear-contaminated water can trigger cell mutations. This could turn normal cells into cancer cells or make existing cancer cells more aggressive [32]. The unique nature of the interactions between nuclear-contaminated water and cancer cells, which may be due to differences in contaminant composition, structural characteristics, and the unique molecular pathways of MDA-MB-231 cells.

Overall, this study shows the potential dangers of nuclear-contaminated water. Future research should focus on finding out exactly which parts of nuclear-contaminated water are toxic and elucidating the molecular mechanisms behind these effects. Conducting comprehensive risk assessments and implementing appropriate regulatory measures are crucial steps to address the environmental and health impacts of nuclear-contaminated water. By doing so, we can better understand and mitigate the threats posed by nuclear - contaminated water, not only for the well-being of human health but also for the protection of the environment.

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