Radiation-induced Bystander Fibroblasts Both Reduce and Amplify Micronuclei Induction through the Reciprocal Bystander Effect and the Secondary Bystander Effect

By

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Abstract

Aside from directly causing DNA damage, the traversal of radiation through cells also induces the bystander effect, which is the biological response of unirradiated cells that are neighboring or sharing medium with the irradiated cells. Although the mechanisms through which irradiated cells send signals to the bystander cells are not well understood, the bystander effect could potentially have clinical relevance or play a significant role in low dose radiation environments. The research in this thesis focuses on the ability of the bystander cells to influence the behavior of cells that share medium with them, which can be separated into three categories: unirradiated cells, irradiated cells, and the original irradiated cells that caused the bystander effect. These can be considered the "secondary bystanders." Human AG01522 fibroblasts were irradiated with 250 kVp X-rays and co-cultured with unirradiated fibroblasts to generate bystander cells, which were then co-cultured with one of the three types of secondary bystander cells. The micronucleus assay was used to analyze the amount of chromosome aberrations present. In the unirradiated secondary bystander population, an increase in percentage of binucleated cells with micronuclei from the background level to approximately the level of the primary bystander cells was observed, indicating that bystander cells can send damaging signals. The data also showed that there was a lower frequency of micronuclei formation in the irradiated population with bystander inserts in comparison to irradiated populations without bystanders. However, there were no conclusive data on the effect of the bystander cells on other irradiated cells. Overall, the results suggest that bystander fibroblasts are capable of sending both detrimental and beneficial signals and can induce a range of behaviors in other cells.

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1. Introduction and Background

Bridging the subjects of nuclear physics and biology, radiobiology is the study of the effect of radiation on organisms. The pioneers in the field of nuclear science, such as Becquerel and the Curies, were also the first to note the biological consequences of working with radioactive materials that emit ionizing radiation (1). Radiation interacts with matter by transferring energy to subatomic particles, atoms, and molecules via different mechanisms depending on the type of radiation, and the negative side effects experienced by those nuclear physicists were the result of ionizing radiation traversing through their cells, depositing energy along its path, and disrupting normal cell functions. The energy from the ionizing radiation can promote chemical reactions that can be unfavorable, creating reactive species that could be harmful to cells. The radiation can also directly interact with the genetic material and alter the DNA by producing strand breaks, base deletions, and other types of changes. The cells with DNA damage are likely to undergo mitotic death, but occasionally, the damage becomes a mutation that leads to cancer. This phenomenon has been studied extensively throughout the past century, and scientists and doctors can now quite accurately quantify dose to humans in order to recommend dose limits for radiation workers and also to use radiation as a form of therapy to kill tumor cells (1, 2).

However, it had been reported for several decades that even cells not directly traversed by radiation were impacted by the radiation's presence if the cells were in proximity to irradiated cells (2). This observation was coined as the radiation-induced bystander effect, but was not studied extensively until the early 1990s (2, 3). Since then, much effort has been put into characterizing the bystander effect, looking at its dependence on environmental factors and investigating the mechanisms behind it. Despite the progress, the consequences of the bystander effect still cannot be taken into account for the purposes of quantifying biological effects of radiation dose. There are still many aspects of the bystander responses that have not been explained, and it is also possible

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that the bystander cells themselves are capable of releasing signals to other cells, creating a "secondary bystander effect." This project examines the ability of the bystander cells to communicate to cells with which they share medium. There are three parts of the project, each corresponding to a different category of cells that is used as the "secondary bystanders," which are unirradiated cells, newly irradiated cells, and the original irradiated cells that initiated the primary bystander effect. The result of this research will help gauge whether the affected area could be larger than just irradiated cells and whether the bystander process could be beneficial to the initially irradiated cells.

1.1. Radiation-induced Bystander Effect

As mentioned previously, the radiation-induced bystander effect is defined as the biological response of cells near or sharing medium with irradiated cells. Although it may seem simple from its definition, the exact "response" of the cells can be manifest in many types of cellular changes and is dependent on many factors, such as the type and energy of radiation, the dose absorbed, and the time after irradiation when the cells are co-cultured. Bystander cells may exhibit a range of behaviors that are not present in an average, normally functioning cell, including gene mutation (4, 5), altered gene expression (6, 7), early apoptotic cell killing (8), and DNA damage (9, 10). The bystander response is also not exclusive to only certain kinds of radiation or linear energy transfer (LET) values (11–14). One characteristic of the bystander effect that has been frequently observed is that it increases at lower doses and saturates at higher doses (15, 16).

Recently, research within the topic of the bystander effect has shifted toward studying the responses from a space radiation environment (15). Accurately quantifying biological effects of radiation dose for astronauts in space is crucial as the space radiation environment is very different from that on earth. Radiation in space, which is a combination of galactic cosmic rays (GCR) and

solar particle events (SPE), has a low particle fluence, suggesting that the direct damage in the irradiated cells will be equally important as the non-targeted effects, one of which is the bystander effect (15, 17–19). Several articles published evidence that bystander responses were detected even when the target cells were irradiated with an extremely low dose (3, 5, 12, 20), but there is a lack of literature on the potential for the bystander cells to amplify the damage by extending the original signal.

1.1.1. Previous Research on the Bystander Effect

Much of the current research focuses on examining how the bystander signal is transmitted between cells (15). While experimental evidence has been published supporting a number of models of the mechanism, the chemical agents that are released by the irradiated cell and taken up by the bystanders are still under investigation (6, 7, 21–28). Two of the recognized models of how the process occurs suggest that it can happen through the release of signals into the medium (12, 21, 23) or across the gap junctions that connect the immediately adjacent cells (10, 22, 29). This project utilizes the former model and induces the bystander effect by co-culturing the irradiated and unirradiated populations such that they share medium but are not touching. As the bystander effect occurring through medium-mediated diffusion has been demonstrated before, medium-sharing is a system often used in studies within the bystander field. It has been speculated that the agents exchanged between the two populations during medium sharing can be a number of compounds, including reactive oxygen species (ROS, 21–23), reactive nitrogen species (RNS, 24, 25, 30, 31), calcium (26, 32), and cytokines (6, 27, 33), and that the process could be facilitated by proteins and enzymes as well (7, 28, 34). Aside from medium-sharing and medium transferring, other popular methods include the use of microbeam facilities, which allow the scientists to target single cells or even portions within a cell for irradiation (16, 20, 35, 36).

Since many aspects of a bystander experiment can be changed, research regarding factors that may affect the bystander response has yielded a large range of results (15, 18). Most studies are still focused on the interactions between the irradiated and the bystander cells, specifically the signaling process from the irradiated to the bystander. However, some researchers have proposed the possibility that the bystander cells could initiate interactions with other cells and send signals of their own. It has been suggested that bystander cells could, in a sense, forward a signal that was received from the original irradiated cell, propagating the bystander response and increasing the affected area (37). Furthermore, bystander cells could also have the capability to signal to irradiated cells, which could potentially be a "rescuing" mechanism. A specific case is the "reciprocal" effect of the bystander cells, where signals are sent back to the original irradiated cell (38–40). This phenomenon has been observed in the data of several published articles. However, because different radiation types, cell lines, and endpoints to measure cell response had been used, no general conclusions could be made about the existence of such behavior for circumstances outside of those as described in the experiments.

1.2. Research Goals

This project seeks to verify whether the effects as mentioned above can be observed in human fibroblasts (AG01522), with the target cells being irradiated with X-rays. Specifically, the main goal is to assess whether it is possible for the radiation-induced bystander fibroblasts to influence the level of micronuclei formation in the cells that share medium with them. These bystander cells are co-cultured with unirradiated cells, newly irradiated cells, or the initial irradiated cells. DNA damage in those three sets of cells is quantified by recording the percentage of cells with micronuclei. This data, as compared to the controls, determine the magnitude of these "secondary bystander effects" and whether they are beneficial or detrimental.

2. Methods and Materials

In the following subsections, the materials have been purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise specified.

2.1. Endpoint and Assay

For this project, the cytokinesis-block micronucleus assay was used as an endpoint in order to measure DNA damage quantitatively. Micronuclei (MN) result from chromosomal aberrations, in this case caused by ionizing radiation. When the radiation is incident upon DNA, it can cause double strand breaks and therefore fragmentation of chromosomes. If mis-repaired or left unrepaired, the fragments are not included in the daughter cells upon mitotic division and are left to form micronuclei found in the cytoplasm after the daughter cells have formed. Therefore, cells with micronuclei can only be scored in binucleated (BN) cells to ensure that only cells that have experienced nuclear division are counted (41). Binucleated cells are created due to the addition of cytochalasin-B, an inhibiting compound that affects the formation of microfilaments, thereby preventing cytokinesis (42). This allows the daughter nuclei and micronuclei to remain in close proximity with one another. Figure 1 shown below is a sample image showing binucleated cells with and without micronuclei taken from one of the slides used in this experiment
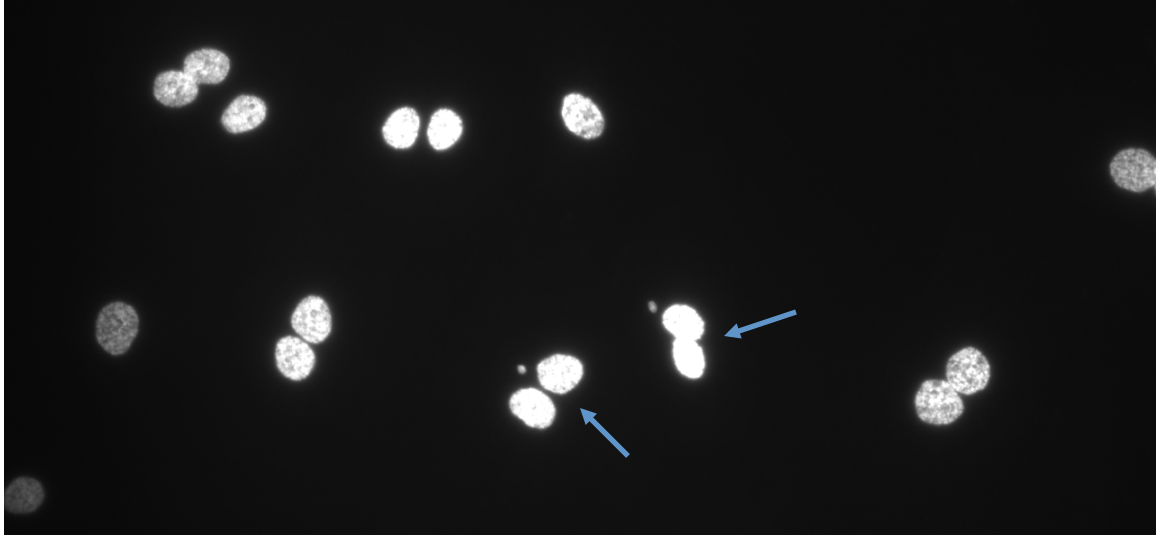
Figure 1: A microscope image of AG01522 after the cytokinesis-block micronucleus assay was performed, taken from a sample used in this experiment. The cells were stained with a fluorescent dye (DAPI), and the percentage of binucleated cells with micronuclei was counted. The arrows point to binucleated cells with micronuclei.

Human fibroblasts (AG01522) were cultured until confluent and seeded on coverslips in multiwell plates or in inserts for bystander cells. The cells were allowed to attach and grow in 3 mL of medium for twenty-four hours before the medium was aspirated for irradiation. After irradiation, 4 mL of medium was reintroduced into the wells along with inserts containing the unirradiated cells. The irradiated and unirradiated cells share medium and are co-cultured for two hours before the unirradiated bystander cells are then moved to co-culture with other cells, which are either also unirradiated or newly irradiated. At the same time, 6 μL of the cytochalasin-B (cyto-B) solution is added into the wells of the irradiated cells. Similarly, the same amount was added to the wells of the "secondary bystanders" two hours after the bystanders were inserted. The cyto-B solution is solid cytochalasin-B compound diluted in liquid dimethyl sulfoxide (DMSO) to 1 μg/μL, and 6 μL were added to the wells to achieve a concentration of 1.5 μg/mL. Starting from the moment when co-culturing began, the cells were incubated for seventy-two hours before fixing. The coverslips with

cells attached were rinsed with phosphate-buffered saline solution (PBS) twice before being fixed in

methanol: acetic acid (3:1, v/v). Then the coverslips were allowed to dry overnight in the fixing

solution before being rehydrated with PBS and stained and mounted onto glass microscope slides

(Fisher Scientific, Pittsburgh, PA) with Vectashield Mounting Medium with DAPI (1.5 μg/mL,

Vector Laboratories, Burlingame, CA). DAPI is the compound 4', 6-diamidimo-2-phenylindole,

which binds to nuclear material and fluoresces blue (43). Using a fluorescence microscope, 500

binucleated cells were scored from each slide and the percentage of those cells with micronuclei

under each treatment was recorded.

2.2. Equipment

2.2.1. Irradiation Source

After media were aspirated from the wells, the cells were irradiated with the 250 kVp X-ray

machine (Siemens Stabilipan 2) operated at 13mA at the Cellular and Molecular Radiation Oncology

Laboratory at the Massachusetts General Hospital in the Charlestown Navy Yard. Irradiations were

performed at room temperature and with a dose rate of 153.8 cGy per minute. At this rate, it took

0.35 minutes and 1.33 minutes to deliver a dose of 0.5 Gy and 2 Gy respectively to a six well plate.

2.2.2. Cell Line and Culture

The AG01522 human diploid skin fibroblast cells used in this experiment were obtained

from the Genetic Cell Repository at the Coriell Institute for Medical Research in Camden, NJ. The

cells were cultured with medium in cell culture flasks (Falcon, Durham, NC) until confluent in an

incubator (Thermo Fisher Scientific, Waltham, MA) that maintained a humidified environment at

37° C with 95% air and 5% CO_2. No cells beyond passage number eight were used. Because cells'
radiation sensitivity is dependent upon the cell cycle, the cells were seeded only after confluency was
reached in order to align their locations in the cell cycle.

The medium is comprised of alpha-modified Minimum Essential Medium Eagle with
penicillin-streptomycin, L-glutamine (L-glut), non-essential amino acids, HEPES (4-(2-
hydroxyethyl)-1-piperazineethanesulfonic acid), and 20% fetal bovine serum (FBS, HyClone, Logan,
UT). One microscope cover glass (coverslip, Fisher Scientific) was placed in each well and insert
prior to seeding. The cells were harvested with trypsin, counted, and then plated with 3mL of
medium at $8x10^4$ cells per well, $4x10^4$ cells per insert.

Each tissue-culture treated polystyrene companion plate (Falcon) had six wells, and a cell
culture insert (Falcon) could be added to each well such that the cells in the wells and inserts were
sharing medium but in direct contact. The insert dishes had polyethylene terephthalate (PET)
membranes with 1μm pores stretched across the bottom, allowing solutes within the shared medium
between the insert and the well to pass freely but preventing the two populations of cells from
mixing.

2.3. Procedures

2.3.1. Secondary bystander effect on unirradiated cells

To examine the effect of the bystander cells on unirradiated cells, the percentage of
binucleated cells with micronuclei in unirradiated cells co-cultured with radiation-induced bystanders
were compared to that of the primary bystander cells. Cells cultured in wells were irradiated at either
0.5 Gy or at 2 Gy; an unirradiated sample was used as control. Following the irradiation, medium
was immediately reintroduced into each well, and the inserts were added. After the two populations

were co-cultured for two hours, the media in the inserts were aspirated, and the inserts were moved to wells with unirradiated cells. The MN assay was performed on the irradiated cells, the initial bystander cells, and the secondary bystander unirradiated cells.
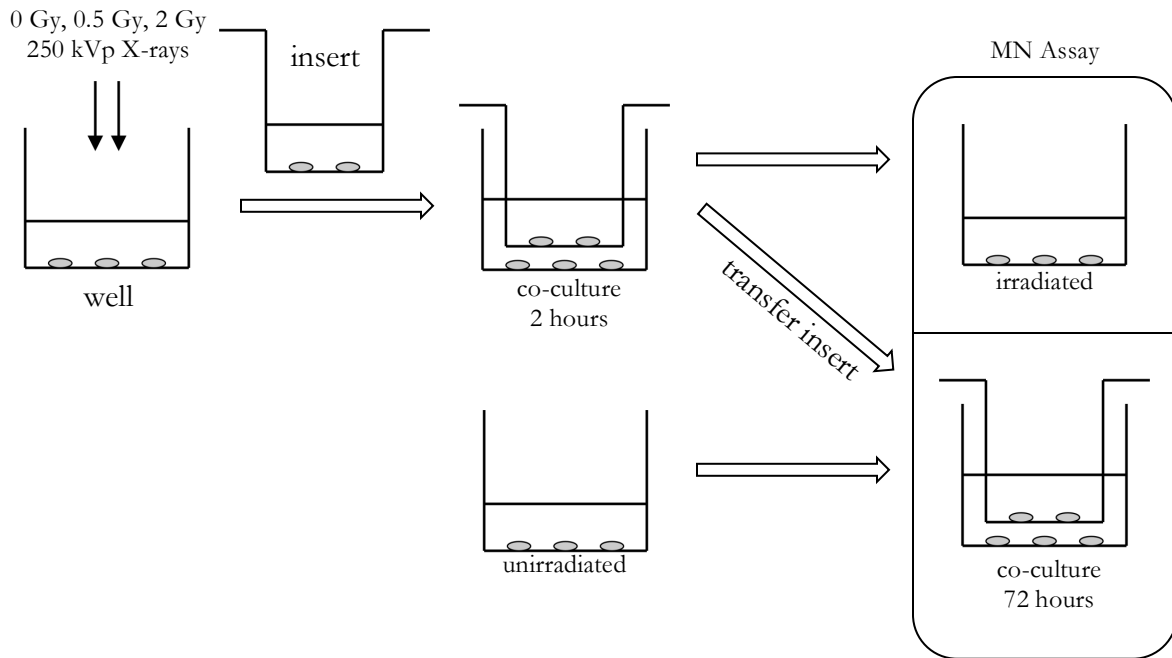

Figure 2: Scheme of insert transferring and co-culture procedure used in experiment to investigate the effect of bystander cells on unirradiated cells.

2.3.2. Effect of bystander cells on other irradiated cells

An insert transfer and co-culture system similar to the one in the previous experiment was used to determine the effect of bystander cells on other irradiated cells. In order to compare the DNA damage in irradiated, secondary bystander cells to the amount of damage in irradiated cells with no bystanders, primary bystander cells were first co-cultured with the initially irradiated cells and then transferred to share medium with freshly irradiated cells. The initial irradiated population was irradiated at 0.5 Gy and 2 Gy, with a 0 Gy sample included as control, while the secondary

irradiated population was irradiated only at 0.5 Gy. Therefore, the inserts were bystanders of

unirradiated cells, cells irradiated at 0.5 Gy, and cells irradiated at 2 Gy, but they were all co-cultured

for a second time with newly irradiated cells at 0.5 Gy. The MN assay was performed on the

secondary irradiated cells with bystander inserts and irradiated cells with no inserts.
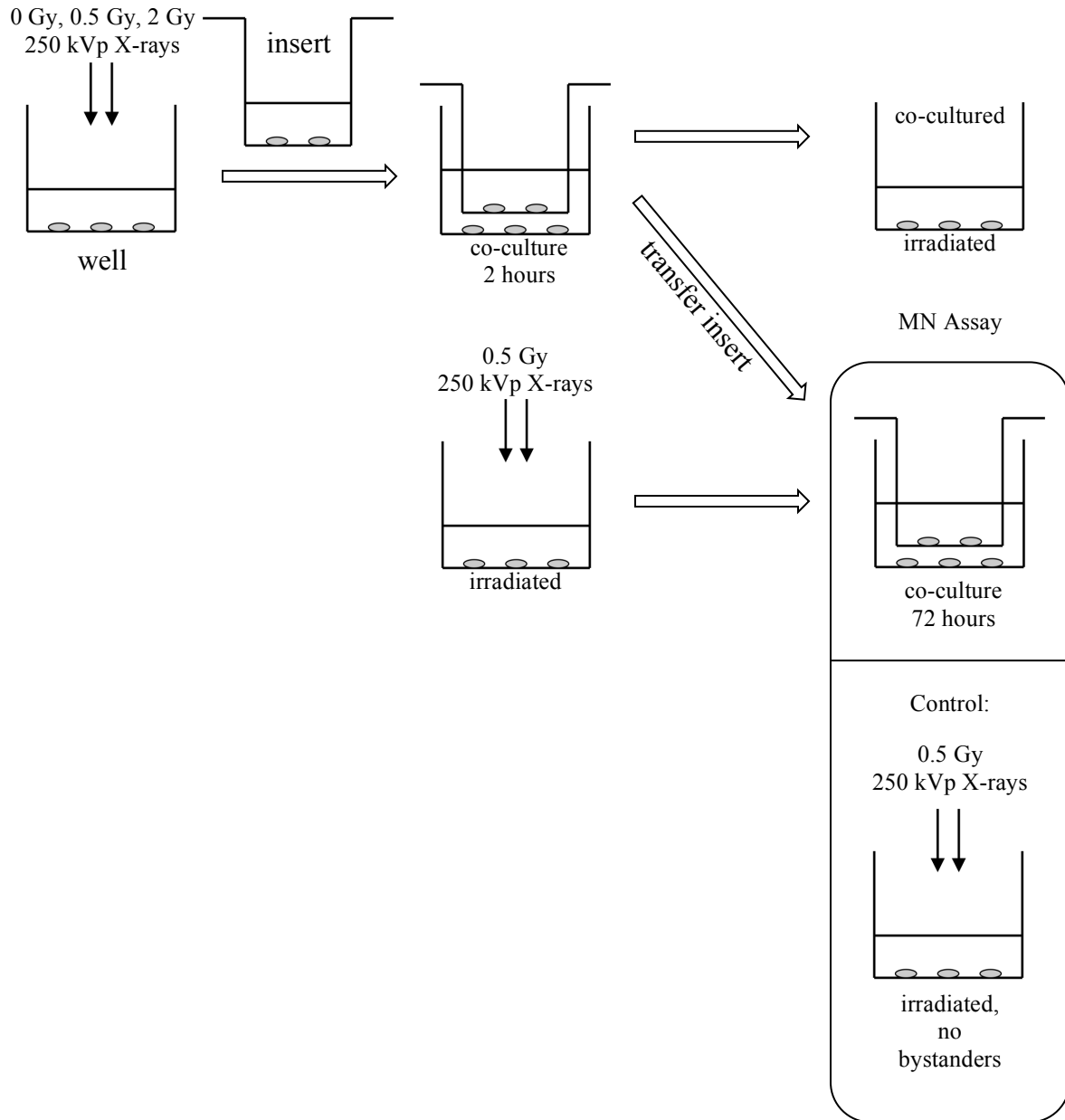

Figure 3: Scheme of insert transferring and co-culture procedure used in experiment to investigate
the effect of bystander cells on other irradiated cells.

2.3.3. Effect of bystander cells on the initially irradiated cells

In this portion of the experiment, irradiated cells with no bystander cells were compared to irradiated cells with bystander inserts added. For both categories, the cells were irradiated at 0.5 Gy and 2 Gy, and a sample at 0 Gy was used for control. The irradiated cells with bystanders were co-cultured for just two hours before the bystanders were removed. The MN assay was performed on both the irradiated cells co-cultured with the bystanders and the irradiated cells with no inserts added.
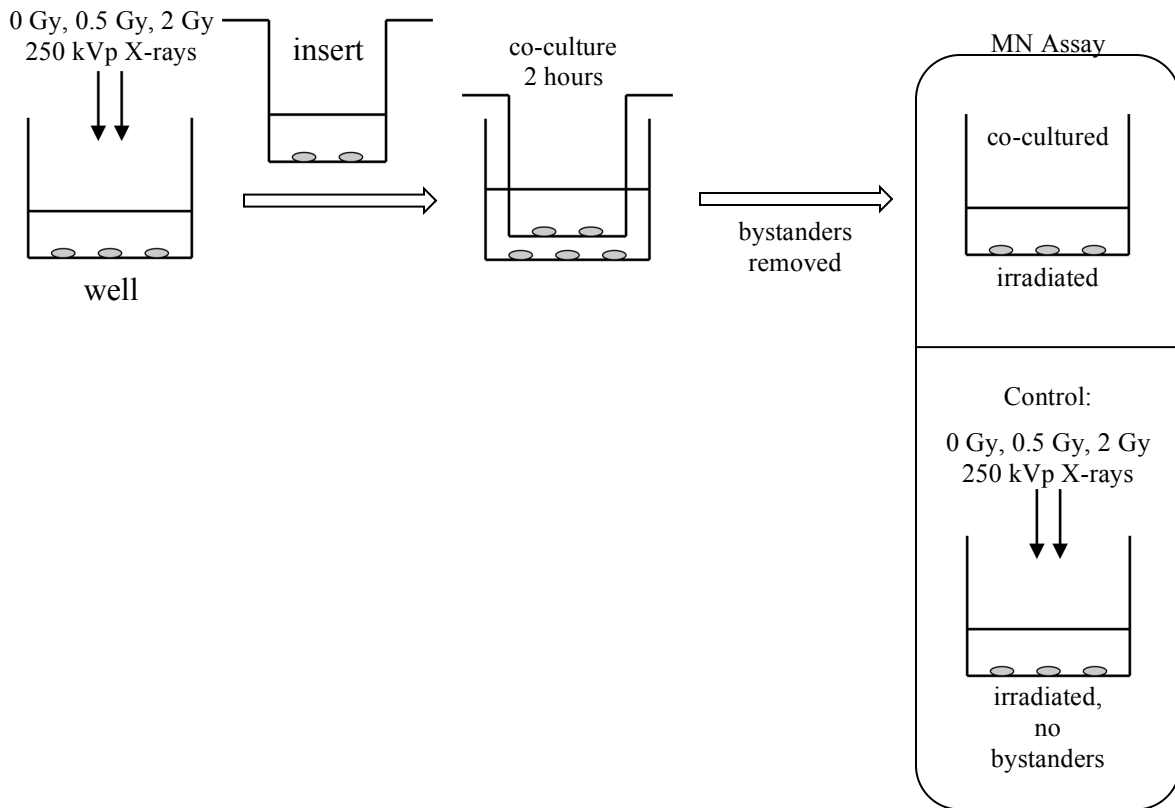

Figure 4: Scheme of co-culture procedure used in experiment to investigate the effect of bystander cells on the initially irradiated cells.

2.4. Statistical Analysis

The results are presented as the mean ± standard error based on the data obtained from at least three separate experiments. A t-test was performed using MATLAB to compare the data set of interest for each of the three effects being investigated. $p \leq 0.05$ was considered significant.

3. Results

The goal of this project is to examine the effect of radiation-induced bystander cells on cells that share medium with them. These cells could be considered "secondary bystander" cells and can be grouped into three distinct categories: unirradiated, irradiated, and the initially irradiated cells that sent the primary bystander signals. Each group can react differently to the presence of bystander cells, and understanding their behaviors is necessary for accurately quantifying the biological effect of ionizing radiation. To simulate the bystander process, a transwell insert co-culture system was used such that two populations with different dose treatments share medium without being in direct contact. The micronuclei assay was performed to determine the amount of DNA damage in each sample, and the data are presented as percent of binucleated cells with micronuclei.

3.1. Secondary bystander effect on unirradiated cells

Figure 5 shows the amount of micronuclei induction present in the unirradiated secondary bystander cells in comparison to the primary bystander cells and the initially irradiated cells. While there is not a significant difference between the samples with 0.5 Gy and 2 Gy induced primary bystanders in the secondary bystander population (p>0.05), they are distinct from the samples with 0 Gy induced primary bystanders. It appears that the 0.5 Gy and 2 Gy induced primary bystanders

caused a 1.6-fold increase in the amount of MN formation in the unirradiated AG01522 cells (p<0.01), such that they exhibit virtually the same magnitude of damage as the primary bystanders.
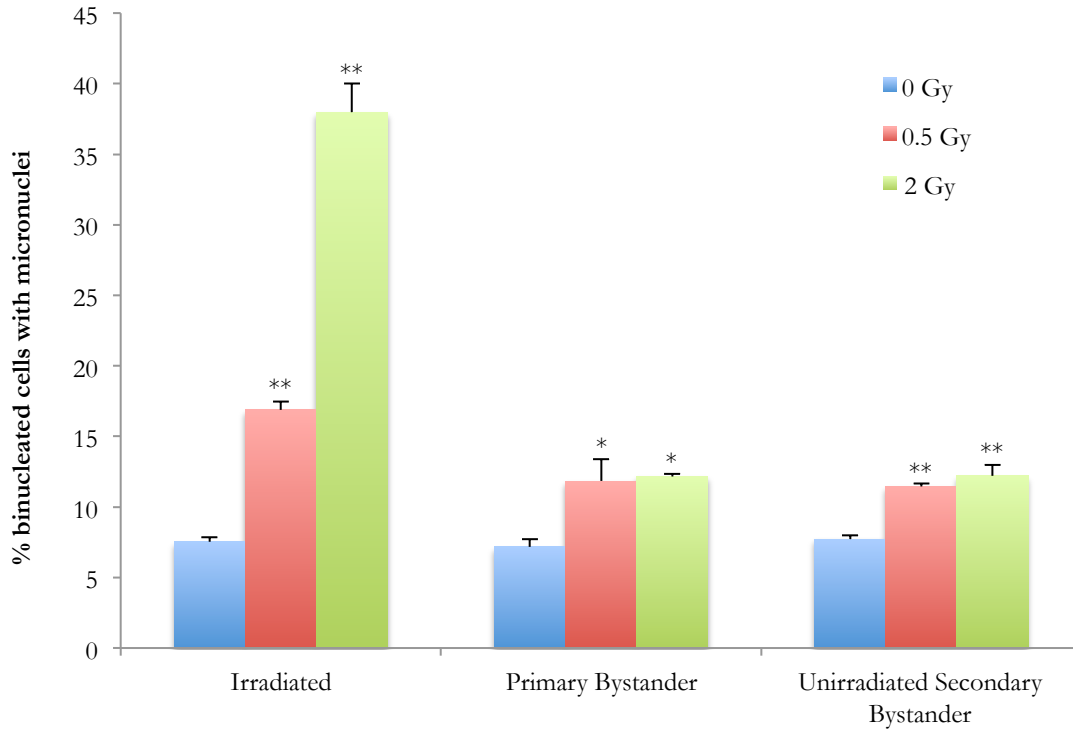

Figure 5: Micronuclei induction in the irradiated population, the primary radiation-induced bystander population, and the unirradiated secondary bystander population. The doses indicated in the legend were delivered to the original irradiated cells. Data represent the means of three separate experiments, and errors bars represent the standard error. An asterisk indicates p<0.05 while two indicates p<0.01, compared to 0 Gy samples in the same category.

3.2. Effect of bystander cells on other irradiated cells

Figure 6 compares the population of secondary bystander irradiated at 0.5 Gy co-cultured with 0 Gy, 0.5 Gy, and 2 Gy induced bystanders with the 0.5 Gy irradiated sample with no bystanders inserted. There seems to be an increasing trend in the frequency of MN formation with increasing doses to the original irradiated cells, and samples with bystander cells inserted saw 5-8% more MN induction when compared to the control sample irradiated at 0.5 Gy with no insert.

However, none of the differences between treatments were statistically significant, with p-values ranging from 0.2 to 0.9.
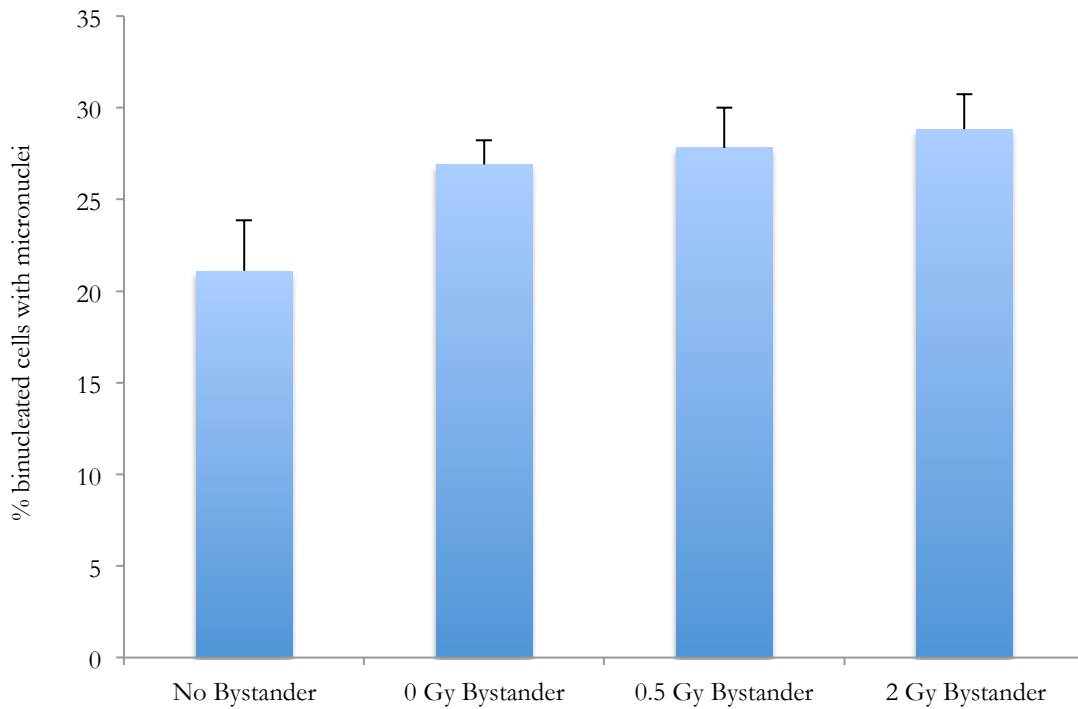

Figure 6: Frequency of micronuclei formation in populations irradiated with 0.5 Gy and then either not co-cultured or co-cultured with bystander cells induced by varying levels of radiation dose. Data represent the means of three separate experiments, and errors bars represent the standard error. An asterisk indicates $p<0.05$ while two indicates $p<0.01$.

3.3. Effect of bystander cells on the initially irradiated cells

Figure 7 shows MN formation in the irradiated cells with and without unirradiated bystander cells. Both groups show similar increasing trends as the dose is increased, but the overall levels in the populations co-cultured with bystander cells appear lower. At 0.5 Gy, the means of MN frequency of the cells with no bystanders inserted is about 4% more than that of the cells co-cultured with bystanders. The largest difference is in the samples irradiated with a dose of 2 Gy, where the means differ by approximately 12%. In fact, the difference between the two groups is

statistically significant at both 0.5 Gy and 2 Gy. This supports the notion that bystander cells can have a positive, "rescue" effect on the initially irradiated cells, which has been hypothesized in previous research (38–40).
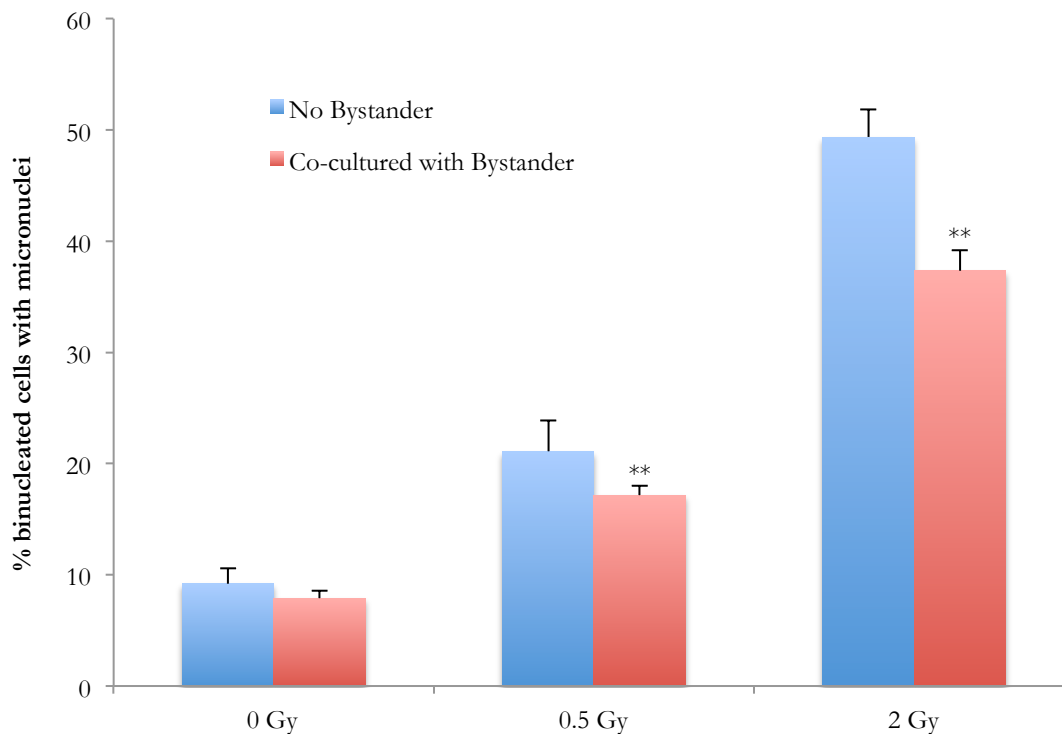

Figure 7: Frequency of micronuclei formation in populations that are unirradiated, irradiated at 0.5 Gy, and irradiated at 2 Gy with and without bystanders. Data represent the means of three separate experiments, and errors bars represent the standard error. An asterisk indicates p<0.05 while two indicates p<0.01.

4. Discussion

Much research has been done on the bystander effect in an attempt to evaluate the role it plays as a part of the biological effects caused by ionizing radiation. Its discovery highlighted a group of cellular responses that are not directly caused by the radiation, often called the "non-targeted effects." The bystander effect is now understood to be a consequence of irradiated cells

sending chemical signals to cells that are nearby, but there is a lack of studies on the bystanders'

abilities to also communicate with other cells. This project investigates whether such abilities exist

and the subsequent behaviors induced in the "secondary bystander" cells.

4.1. Secondary bystander effects on unirradiated cells

One of the abilities examined was the potential for bystander cells to propagate the signals

sent by the original irradiated cell and generate more DNA damage in other unirradiated cells. The

results (Figure 5) indicate that there is indeed more chromosome aberrations as shown through the

increased frequency of binucleated cells with micronuclei in unirradiated cells co-cultured with

bystander cells as compared to the background level. Moreover, the level of micronuclei induction

seen in the secondary bystanders is roughly equal to the level seen in the primary bystanders. It is

unclear whether the severity of the effect is dependent upon the dose of the radiation that induced

the primary bystander effect. The existence of a "secondary bystander" effect suggests that the

overall non-targeted responses caused by the chemical signals released by cells traversed by radiation

are not well quantified. It could be possible that the secondary bystanders could also send signals

that result in more DNA damage, creating a chain of events. The affected area could also be larger

than previously expected.

4.2. Effect of bystander cells on other irradiated cells

This study also investigated whether bystander cells could influence other irradiated cells in

the shared medium. Data suggest that for AG01522 fibroblasts, there is no observed significant

difference between the amount of micronuclei seen in populations irradiated with 0.5 Gy regardless

of whether the cells were co-cultured with other cells (Figure 6). There were also no significant

changes in micronuclei count between the bystander cells induced with different doses of radiation. Although this set of data indicates that there were likely no effects of the bystander cells on the irradiated cells in this experimental set up, it does not conclude that such an effect will not exist at all. Only one dose for the irradiated, secondary bystander cells was used in this particular experiment, and the cells were co-cultured with the bystander cells immediately after irradiation. This most likely caused the data for the samples with 0 Gy and 0.5 Gy induced bystanders to be quite similar despite the significant difference in amount of DNA damage generally observed between the bystanders at those dose levels. In previous studies, it was shown that bystander responses could be detected even if the cells were not co-cultured for several hours after the initial irradiation (14). Therefore, a plausible explanation for the unexpectedly similar data is that the unirradiated bystanders received signals from the 0.5 Gy irradiated "secondary bystanders" and became virtually the same as the 0.5 Gy induced bystanders. Since the experimental procedures relied on the method of medium sharing, there could have been an exchange of signals between the two populations. In order to examine the effect with chemicals released only from the bystanders, a medium transferring technique could be considered.

4.3. Effect of bystander cells on the initially irradiated cells

In addition to the two mentioned above, the third effect this project aimed to observe was the bystander cells' potential ability to cause a positive, "rescue" effect in the original irradiated cells. This phenomenon had been detected in previous experiments done with varying kinds of cell lines, radiation types, and endpoints (38-40). The data from this project (Figure 7) indicate that this "reciprocal" effect indeed exists for AG01522 fibroblasts when measuring DNA damage using the micronucleus assay. Earlier in section 4.1, it was noted that bystander cells can create more bystander cells by propagating a damaging signal. Since bystander cells are capable to cause both

detrimental and beneficial responses, a possible explanation is that the overall bystander effect exists to limit excessive damage. Severe damage as experienced by the initially irradiated cell can be slightly mitigated by the bystanders, but as a result, the bystander cells and other nearby cells will sustain some low level damage. Whether the overall biological effect is positive or negative is unclear.

5. Further Research

The results from the three experiments in this project show that there is still much to be learned about the bystander effect. Since bystander responses can dependent on many variables such as cell line, type of radiation, and endpoint, similar experiments can be done varying those factors to determine whether bystander cells can cause other effects under all conditions.

Regarding the secondary bystander effect, studies should be performed to measure the capability of the bystander cells to propagate the signal by co-culturing the secondary bystander cells with unirradiated cells to create tertiary bystander cells and so on. The mechanism used in this project was co-culturing and medium-sharing, but an experiment to observe the secondary bystander effect using gap junctions as the method of communication between cells can also be done. Although it can be concluded that a secondary bystander effect could exist from the data from this project, the results create more questions than answers. The mechanisms and agents behind the bystander effect remain unclear, and it is possible that the secondary bystander effect has different characteristics and is a consequence of a different set of mechanisms and chemical agents.

The data from this project appeared inconclusive when examining the ability of bystander cells to affect other irradiated cells. It is unknown as to whether this is an outcome of poor experimental design or a true lack of the phenomenon. Further studies on the subject should allow the transfer of solutes in the medium to occur in only one direction, from the bystander cells to the irradiated cells, in order to maintain the integrity of the unirradiated bystander cells. This can be

done through media transferring. Various doses can be given to the recipient irradiated cells, which can then receive media from unirradiated bystander cells induced by irradiated cells of varying dose as well. Another factor that can influence the results of similar experiments is time. Given that irradiated cells no longer send bystander signals around 6 hours after irradiation (14), perhaps experiments using the medium-transfer technique can begin the co-culturing process after the 6 hours to ensure that the recipient irradiated cells are not altering the bystander cells.

Researchers should continue to determine whether the reciprocal effect could be observed under all conditions and with various endpoints. Since it is a beneficial process, its mechanisms and the signals used are likely to be different from what is known about how the bystander process occurs. If discovered, the mechanisms could provide valuable insight into how cells reduce radiation damage.

6. Conclusion

The three effects investigated in this project all contribute to the overall understanding of the bystander effect and how it can initiate other responses and behaviors. Information from studies done on this subject allow scientists to better assess the biological effects of ionizing radiation and its non-targeted responses, which are relevant to a number of fields, including protection for radiation workers and radiation therapy and oncology. Despite having inconclusive data regarding the ability of bystander cells to communicate to other irradiated cells, the results from this experiment does show that bystander fibroblasts have the ability to be both detrimental and beneficial when induced by cells irradiated with X-rays. They have the capability to amplify the DNA damaging signal from irradiated cells and increase the level of micronuclei induction in unirradiated cells when sharing a common medium. They also can reduce the frequency of micronuclei formation in the originally irradiated cells. There are still many unknowns regarding the

three effects, and more research must be done before these observations can be used clinically or in

the radiation protection industry.

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8. List of Figures