Cellular responses to UVA as a source of oxidative stress

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Background

Oxidation and reduction reactions are important in driving many processes in living organisms. They are chemical reactions that involve the changes in oxidation number between the reactants to products. Pro-oxidants and antioxidants are reagents that drive these reactions, and a balance of them is essential in living organisms. However, when Reactive Oxygen Species, a type of pro-oxidant exceeds the antioxidant capacity, oxidative stress is generated. Reactive Oxygen Species (ROS) in excess can generate oxidative stress that can lead to some major diseases such as cancer, cardiovascular disease, and neurodegenerative diseases.

There are many ways to generate ROS or oxidative stress such as environmental pollution and various forms of radiation. UV light reaches earth in two main forms: UVA (90%) and UVB (10%). Much more is known about UVB and its deleterious effects, such as skin cancer, photodamage, and DNA lesions. Because DNA absorbs at the wavelength of UVB light, when the cells or tissues are irradiated, DNA and other endogeneous photosensitizers in the cells can absorb and mutate. Hence, most of the UV protections used today, such as sunscreens are really focused on blocking out UVB (280-320nm). However, skin cancer and various problems are still prevalent even with such protection. This shows that there are other factors that can contribute to these problems and diseases, such as UVA radiation since about 90% of the UV light that reaches earth's surface is in this form after all. UVA has a slightly higher wavelength (330-395nm) and is known to induce cellular oxidative stress, which can eventually lead to cancer.

It is previously thought that in order to generate oxidative stress, cells must be directly irradiated. However, previous studies in the Wellman Lab at Massachusetts General Hospital have shown that with ionizing radiation and photosensitization, the irradiation not only affects the target cells, but the neighboring cells as well. This effect on the naive neighboring cells is called a bystander effect, in which when cells communicate with one another, the cells and tissues not directly irradiated have also increased in oxidative stress, mutagenesis, and decreased in cell viability and clonogenic survival (the ability of cells to form colonies), the ability of cells to form colonies3. This project investigates whether UVA also generate this bystander effect in cells as well as a look at the mechanisms compared to other means of oxidative insult.

Methods

The preliminary endpoints measured were the cell viability and ROS or oxidative stress levels in cells for both target and bystander cells. The experiment was divided into two main parts: individual or subpopulations and population studies.

Time-Lapse Microscopy

For individual cell studies, EMT-6 breast cancer cells were used. These cells attach to the bottom of the wells, which allows us to monitor and track specific cells. These studies were done with the time-lapse fluorescence microscope. With this microscope, a small region of only a few cells is selected to focus on at a time. Once the target region is selected, the computer can save it such that we can come back to this exact same region at any time. The region is approximately 0.7mm in diameter, and all these cells within the region will be covered by the UVA light. About 5mm away from each region, another region is selected for bystander studies, with no irradiation. Either a 12-well or a 6-well plate was used, and each plate included both a bystander and target region of cells. The cells were approximately 70% confluent in the wells and instead of in regular media, they were incubated in HBSS, which is media that contains mainly salt solution to avoid other variables during irradiation.

Nd:YAG Laser Irradiation

For the population studies, WTK1 human lymphoblastid cells were used. They are suspension cells, and hence do not attach to the bottom of the wells. For bystander experiments, a trans-well system must be used in order to separate the target and bystander cells. This is done by putting bystander cells in a micro-porous insert that sits on top of the target cells in the wells. The pores in the insert allow exchange of cell signals and media. Cells were again incubated in HBSS at about 3 millions cells per milliliter, placed in a 1mm clear square cuvette and irradiated with the Nd:YAG laser.

Cell Viability/Survival

Cell viability in the microscope experiment is measured with the use of propidium iodide (PI), after incubating with 1.7uM of PI. PI is a fluorescent dye that is prohibited by cell membranes, and hence will only go into dead cells. Once it goes into these membranes and into the nucleus, it will show red fluorescence, which can be seen by the microscope. Later, the ratio of red cells to normal, live cells were counted to determine the percent of cells alive. For the WTK1 cells, because there is no microscope used to detect fluorescence, the MTT assay is used to measure cell viability. MTT is a toxic chemical which will go into live cells after 1 hour of incubation, and bind to the mitochondria to form a mitochondria complex. The more mitochondria complexes there are in a sample, the higher the cell viability. In order to detect the amount of mitochondria complexes, DMSO solution is added to dissolve them. Lastly, Spectrmax is used to measure the absorbance of the dissolved solution.

ROS levels/Oxidative Stress

For oxidative stress or ROS levels, the fluorescent dye DCF (2, 7-dichlorodihydrofluorescein diacetate) is used. The green fluorescence of this dye is only turned on when it enters live cells and bind to an active enzyme called oxidase. Oxidase will oxidize the dye and make it fluorescent. The DCF also binds to ROS, and the fluorescent intensity increases with greater levels of ROS.

WTK1 cell viability was measured with different irradiation energies, 0J, 10J, and 20J. Figure 1 shows that the cell viability decreased as the irradiation energies increased. This was true for both direct and bystander cells. The rate seen here for bystander cells is almost the same as for direct cells.

In Figure 2, ROS levels were measured for in both target and bystander cells in the laser experiment. 0J, 10J, and 20J were used and the fluorescence intensity of DCF was taken to measure the oxidative stress.

Figure 3 shows a similar ROS experiment for the time-lapse microcopy. Fluorescence and bright field was taken once every 30 minutes and this experiment ran over night for about 16 hours.

For the ROS experiments, Figures 2 and 3 show that there is an increase in oxidative stress for both direct and bystander cells with increase irradiation energies. In Figure 2a, the ROS levels did not increase after initial DCF incubation for the direct cells, while for the bystander cells, it increased dramatically.

Discussion

From Figure 1, we can see that the bystander cells, which had never been exposed to UVA radiation, showed a decrease in cell viability as well. Hence, we can conclude that UVA does generate a bystander response in terms of cell survival. Previous studies using photosensitization also showed similar results, that the bystander cells near the target cells with higher irradiation energies had much lower (about 15%-30%) cell survival rates1.

From Figures 2 and 3, we can see that oxidative stress in the bystander cells under higher irradiation energies was also increased. This means that there is also a bystander effect in terms of ROS levels. The results also show that for the direct cells, there isn't much change after initial DCF incubation. Studies in photosensitizers showed different results, since for photosensitizers, the DCF intensity level increased after initial incubation, maximize at about 6 hours before it dropped again2. However, for the bystander cells, we see an increase as we increase the incubation time. This is consistent with other oxidative stress experiments because it takes time to generate a bystander effect since cells have to send signals to one another1.

It is also important to note that even though the control cells went up in Figure 2, it is most likely because the cells have all been incubated in HBSS. By placing the cells in HBSS instead of serum media that contains all the essential nutrients, the cells are already under some stress.

In this study, we were able to show that UVA radiation does generate a bystander effect not only in cell viability but also in cellular oxidative stress. High levels of oxidative stress can eventually lead to the development of cancer, not only in the cells that are directly irradiated, but will also spread into neighboring cells. In the future, we would like to investigate the effects of UVA radiation on mutagenesis and clonogenic survival, as well as detailed mechanisms that led to these results compared to other ROS methods.

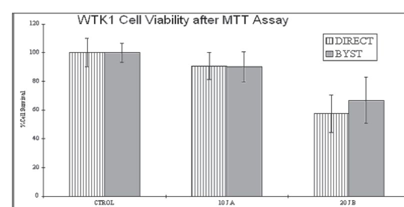

Figure 1

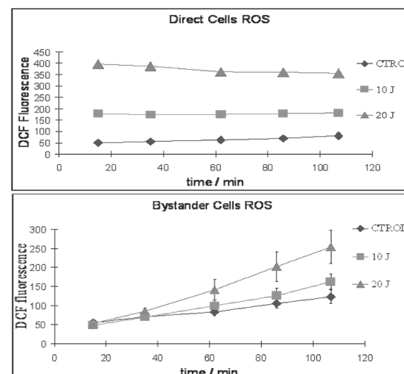

Figure 2

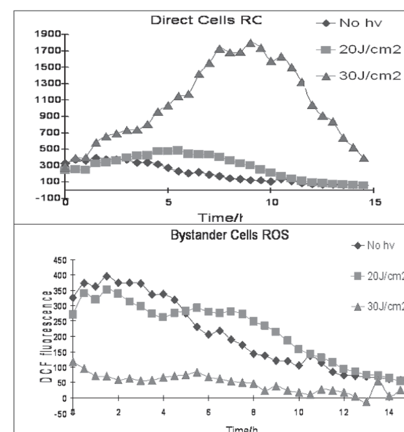

Figure 3

References

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