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The Influence of G-CSF on the Survival Ability of HSCs in High Ionizing Radiation

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Abstract:

In today's constantly developing technology, people are not only using various machine devices such as medical equipment but also being disturbed by the ionizing radiation they bring. How to overcome the impact of ionizing radiation on the human body? This issue was raised as early as after the atomic bomb exploded in 1945. After consulting numerous sources, we speculate that the resistance of human cells to ionizing radiation is influenced by the G-CSF protein. So, we propose that we can enhance the survival probability of adult animal cells in high ionizing radiation environment by changing the content of g-CSF in HSC cells. We hope that such speculations can contribute to future research on ionizing radiation

Keywords: Ionizing Radiation; Granulocyte colony-stimulating molecules

1. Introduction

Following the first atomic bomb detonation in 1945, victims of the Hiroshima and Nagasaki atomic bombs died from infrared radiation by the thousand, which highlights the harmful effects of ionizing radiation (IR) on people's health. Since then, researchers have begun investigating the influence of ionizing radiation, which would probably be on the human body. After Till JE and McCulloch EA discovered the presence of stem cells in the blood in 1961, ionizing radiation was realized to be closely linked to the blood system. Scientists later found that the blood system is extremely vulnerable to ionizing radiation. When the human body is subjected to ionizing radiation, it might develop radiation disease, which is a systemic reaction of the body. Almost all organs and systems undergo pathological changes, but the changes in the nervous system, blood organs, and digestive system are most pronounced. Among them, acute radiation syndrome (ARSs) is the most well-known. It includes clinical manifestations, for example, infection, bleeding, anemia, and so on, that are mainly due to serious bone marrow suppression caused by IR.

The blood progenitor cells (HPCs) and a small amount of blood stem cells (HSCs) undergo apoptosis after exposure to IR, so resulting in acute BM suppression within days. With the continuous deepening of research on ionizing radiation, the corresponding application fields are constantly expanding. As a result, various accidents have emerged due to ionizing radiation. So, it's important to find ways to resistance to IR, which means finding a way to help the blood system resist ionizing radiation. It is believed that the ability of proliferation and differentiation of blood stem cells plays a valuable part in the research of how biological cells survive under high concentrations of ionizing radiation. At the same time, with the further understanding and investigation of blood stem

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cells, its ability is related to three factors: G-CSF / Stat3 / BATF. Among them, granulocyte colony-stimulating molecules(G-CSF) is a glycoprotein, which mainly acts on the proliferation, differentiation, and activation of neutrophil blood cells. At the same time, according to existing scientific papers, G-CSF is the only recommended treatment for radiation victims after undergoing the ageing of blood stem cells induced by electrical radiation. By endorsing this perspective, it follows that the study of what helps blood stem cells survive high concentrations of ionization radiation has focused on the effects of G-CSF on them.

The research about G-CSF has been thriving and gradually become a mainstream field of inquiry. At present, we know that G-CSF under ionizing radiation will activate the dependent differentiation checkpoint to enhance the lymphoid differentiation ability, and further promote the differentiation of blood stem cells and deplete them. G-CSF may impair long-term HSC refilling and self-renewal ability, which may further impair long-term HSC function after IR [1]. More importantly, an extensive search of the literature reveals that people found that HSCs with lymphatic sensitivity after activation of IR G-CSF dependent differentiation control points were found to be more sensitive to differentiation induction than HSCs with myeloid sensitivity inducing myeloid sensitivity in irradiated mice. These results show that induction of HSC differentiation via the G-CSF pathway not only plays a significant role in mediating IR-induced HSC injuries but also contributes to the deviation of the bone marrow system by IR [2]. These results show that induction of HSC differentiation by G-CSF not only plays an important role in mediating IR-induced HSC injuries but also contributes to the IR-induced bone marrow line. What's more, though the G-CSF administration could reduce acute radiation sickness (ARS), it can also aggravate total body irradiation (TBI) induced bone marrow (BM) injury in part by promoting HSC senescence via. Unfortunately, this important area of IR damage as well as G-CSF has not been given sufficient attention. So, this study was conceived against this backdrop to examine the part that G-CSF plays. We want to know if reducing its content in HSCs can help the cells survive in a high IR environment.

2. Methodology

The purpose of this study is to look at the role of G-CSF in blood stem cells (HSCs) under ionizing radiation. And we need technologies to facilitate gene editing. CRISPR is the greatest option; it is a type of gene editing technology that enables scientists to alter DNA, the hereditary information found in humans and practically all other animals. Modifying DNA can alter an organism's features, such as eye color or disease susceptibility. And CRISPR uses bacteria's innate defense mechanism to break DNA at a specified site. At the same time, we know how to use flow cytometry to display the approximate number of HSCs and change the situation of HSCs in IR, to generate fluorescence bias to determine the number of cells. The flow cytometer is a device that automatically analyzes and sorts cells, can quickly measure, store and display a number of important biophysical and biochemical properties of dispersed cells suspended in liquids, and can separate specific subpopulations of cells based on preselected parameter ranges. Also, we need a healthy adult mouse to obtain bone marrow cells from it and randomly divide them into three parts. To avoid the randomness of the experiment, each group needs to have more than two portions of cells placed on the same temperature and nutrient medium. Figure 1 shows the process of experimental

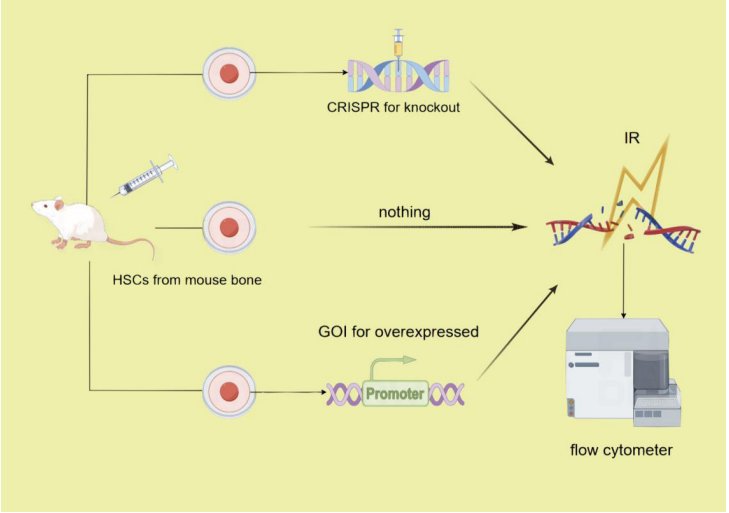

Figure 1. Experimental process

2.1 Question 1: Does G-CSF Have an impact on the resistance of stem cells to IR?

There will be three groups of experiments here, and we will use mouse bone marrow blood stem cells. One group, which is the control group, will not undergo any treatment. We will expose these three groups of cells to medium or high doses of gamma rays (≥ 3.5 Gy). Because in this environment, the reserve and health of HSCs decrease, and HSC self-renewal is impaired. In addition, we also need to treat the other two groups of mouse bone marrow blood stem cells as experimental groups. They should be reduced in one group and increased in another group. First, we will use CRISPR to knock out the gene of one group. The process involves an enzyme that can recognize and cleave specific DNA sequences specified by CRISPR sequences and associated RNA called Case9. After cutting DNA, the cell's repair mechanism would trigger. So, we can obtain a complete DNA strand once again and have a group be reduced. Then we need a group which is increased. We would use the gene of interest (GOI) for overexpression, which could obtain the cDNA of the gene encoding the protein you want to overexpress. We would recombinant suitable promoters with plasmid vectors and then clone them into plasmid vectors using restriction enzymes or similar cloning methods to achieve production until the appropriate density is reached. After all of this work, we can get a group that is overexpressed.
Processing the three groups of cells, they were exposed to gamma rays for a certain period before being removed. At this point, we will use a flow cytometer to confirm whether the presence of G-CSF has an impact on its survival in high-concentration IR. We would use a suitable flow tube for sampling and filtering the cells to prevent cell adhesion and blockage of the pipeline. Then, place the sample to be tested and adjust the flow cytometer settings. After that, we can see the result from the computer, which links to the machine. If it really has an impact, we will see the changes in the cell content measured by the flow cytometer. We predict that the cell content of the first group should be higher than that of the second group while the third group is the lowest.

2.2 Question 2: Will increasing G-CSF gene expression improve the effect of stem cells on IR?

However, due to the lack of resolution of the flow cytometer, we cannot determine whether the content of HSCs has changed through the flow cytometer. So, we need to use new instruments to determine the changes in HSCs. We are considering using the comet assay. It can measure the degree of DNA damage. The more severe the DNA damage, the looser the DNA superhelix structure, resulting in more breakpoints and smaller DNA fragments. As a result, more DNA fragments appear at the tail of the comet, leading to a larger length, region, and fluorescence intensity of the comet tail. By measuring indicators such as length, region, or fluorescence intensity of comet tails, the degree of DNA damage can be quantitatively analyzed. This method is used because when HSCs are exposed to high concentrations of IR, the production of ROS can lead to ageing and damage [2]. There are a total of six steps here to complete the electrophoresis operation.
1. Production. There are three types: "sandwich" gel, double-layer gel, and single-layer gel. 2. cell lysis: the purpose is to remove the cytoplasm, leaving only the DNA in the nucleus, and the lysis time is usually 1 hour. 3. Cracking: after lysis, the double-stranded DNA is opened in an alkaline solution for approximately 60 minutes. 4. Electrophoresis: usually conducted at low voltage (0.5-5V/cm) and short duration (20-30min). If the voltage is too high or the electrophoresis time is too long, comet cells may tail and cause the comet to disappear, while non comet cells may swim out of the tail and form false positive results. On the contrary, if the voltage is too low or the time is too short, DNA fragments are difficult to swim out, and damaged cells have no tails, resulting in false negative results. 5. film reviews: drip ethyl bromide, DAPI, etc., onto a glass slide, cover with a cover glass and observe under a fluorescence microscope. Finally, analysis of experimental results.

3. Conclusion

Through this method, we can clearly observe the changes in HSCs. It can be confirmed that G-CSF has an impact on the resistance of stem cells to IR, but when overexpressed, it does not enhance the cell's resistance, but rather the opposite is true. I think this is not only because, under ionizing radiation, G-CSF is activated to accelerate the differentiation of HSCs, but also because G-CSF treatment increases the number of phenotypic hematopoietic stem cells in the bone marrow, but it can lead to the loss of hematopoietic stem cell refilling and self-renewal activity [3]. That's why when the content of G-CSF increases, the resistance of HSCs actually decreases. Of course, further experiments are needed to verify the expression of HSCs by specific receptors of G-CSF in the IR environment.

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