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To explore the viability of hematopoietic stem cells in high doses of ionizing radiation

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Abstract:

Excessive exposure to radiation poses a significant threat to the integrity of our hematopoietic stem cells, which are essential for blood formation. The primary objective of our research is to safeguard the viability of these cells against the onslaught of high-dose radiation. Despite the critical nature of this issue, current literature lacks an in-depth investigation into the mechanisms by which hematopoietic stem cells respond to such intense radiation. To address this gap, we have embarked on a study to modulate the expression levels of the STAT3 protein within hematopoietic stem cells. By employing both gene knockout and overexpression techniques, we aim to elucidate the role of STAT3 in the survival of these cells post-radiation exposure. Our methodology will involve a meticulous enumeration of surviving cells to assess whether the modulation of STAT3 confers a protective advantage against radiation-induced damage.

Keywords: STAT3, hematopoietic stem cells, ionizing radiation

1. Introduction

Upon meticulous review of the literature, we have uncovered that the Stat3 signaling pathway is not only pivotal in initiating the differentiation of hematopoietic stem cells(HSCs) but also plays a critical role in orchestrating the cellular repair mechanisms following exposure to high- dose radiation (Figure 1) [1]. This underscores the imperative need for a comprehensive grasp of the intrinsic properties of HSCs and the multifaceted role of the STAT3 protein before advancing further in this research domain.

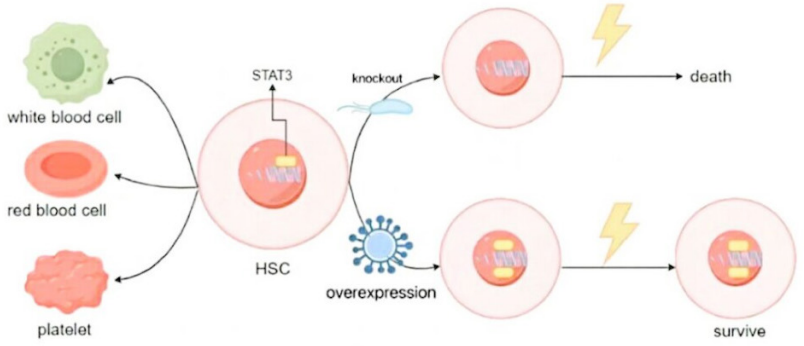

Figure 1. STAT3 Signaling Pathway in Hematopoietic Stem Cells and Its Role in Cellular Repair Mechanisms Post-Radiation Exposure

Hematopoietic stem cell, with their extensive research history, profound understanding, and advanced experimental methodologies, are at the forefront of stem cell research [2]. These cells, produced in the bone marrow of adults, are endowed with the remarkable capacity for self-renewal and multilineage differentiation. They are capable of generating a diverse spectrum of blood cells, including erythrocytes, leukocytes, and platelets, thereby sustaining the perpetual renewal and metabolic functions of the blood system and facilitating the regeneration and repair processes post-injury. The embryonic phase witnesses a robust proliferation of HSCs, which subsequently transitions to a state of relative quiescence as individuals reach puberty.

Signal transducers and transcriptional activators3 (STAT3) are integral cytoplasmic proteins that couple with tyrosine phosphorylation signal channels [3-5]. The unchecked activation of STAT3 can precipitate cellular dysregulation, leading to abnormal proliferation and apoptosis, and may contribute to the genesis and progression of malignancies. Anomalously elevated levels of STAT3 expression have been detected across various tumor types, implicating its significant role in oncogenic processes.

We aim to harness the power of CRISPR-Cas9 gene-editing technology and transfection methodologies to modulate the expression levels of the Stat3 gene, either by gene knockout or overexpression.

CRISPR-Cas9, derived from the adaptive immune systems of bacteria and archaea, utilizes the precision of RNA-guided DNA targeting to facilitate the Cas proteins in executing gene cuts and modifications [6-7]. The versatility of the CRISPR system has expanded dramatically with the discovery and engineering of diverse Cas proteins, along with the identification of a wide array of programmable guide RNAs, solidifying CRISPR's role as a potent tool in molecular diagnostics and therapeutics.

Transfection represents a sophisticated suite of techniques designed to introduce exogenous genetic material into cells. As our understanding of gene and protein functions has deepened, transfection has become a cornerstone method frequently employed in laboratory settings. Transfection methods are categorized into physical, chemical, and biological approaches. Examples of physical transfection include electroporation, microinjection, and gene gun, which introduce genes into cells through mechanical means [8]. Chemical transfection encompasses a spectrum of techniques, such as the traditional calcium phosphate co-precipitation method, liposome-mediated delivery, and various cationic-mediated approaches. Biological transfection ranges from the more rudimentary protoplast transfection to the highly efficient and low cytotoxic virus-mediated transfection techniques. The latter stands out as the most effective method due to its high transfection efficiency and minimal impact on cell viability.

The survival count will serve as a pivotal indicator of the protective or detrimental effects of altered STAT3 expression levels on hematopoietic stem cells under the stress of high-dose radiation.

2. Method

2.1 Objective1: To evaluate the impact of STAT3 gene knockout on IR resistance of stem cells

Rationale: In order to elucidate the role of STAT3 in the IR resistance of stem cells, we will undertake a series of in vitro experiments. These experiments aim to compare the survival rates of STAT3 knockout stem cells with those of normal stem cells following exposure to high doses of radiation, thereby revealing the specific influence of STAT3 on IR resistance of stem cells.

Methods: We have discovered that the SH2 domain of STAT3 is pivotal in the mediation of various signaling pathways [9]. To investigate this, two cohorts of hematopoietic stem cells (HSCs) were prepared: one underwent gene knockout via the CRISPR-Cas9 technique, while the

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other served as a control group without any treatment.

Gene Expression Analysis: Western Blot analysis was employed to monitor the expression levels of genes associated with hematopoietic stem cell function before and after irradiation. This was done to confirm the successful knockout of the STAT3 gene and to assess its impact on the cellular response to stress.

Radiation Treatment: Both groups of HSCs were subjected to high doses of ionizing radiation. Meticulous records were kept regarding the radiation dosage and exposure duration to ensure standardized experimental conditions.

DNA Damage Detection: The extent of DNA damage in the irradiated HSCs was evaluated using single- cell gel electrophoresis [10], commonly known as comet assay, or by assessing the formation of γ-H2AX foci, a recognized marker of DNA double-strand breaks.

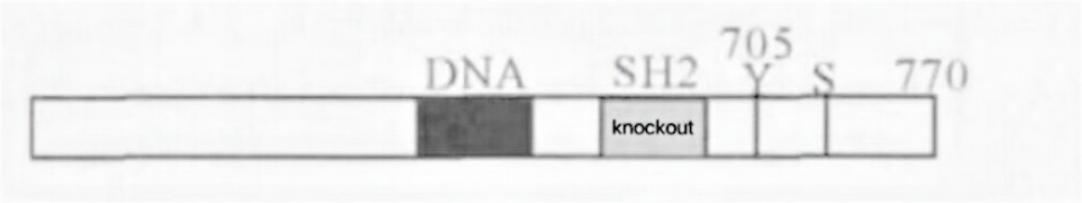

Figure 2. Effect of STAT3 Gene Knockout and Overexpression on Hematopoietic Stem Cell Survival Post-High-Dose Radiation

Expected outcome: Based on our preliminary findings, we anticipate that the HSCs with the STAT3 knockout will exhibit a higher susceptibility to radiation-induced cell death at high doses compared to the untreated control group (Figure 2). We hypothesize that the absence of the STAT3 gene will compromise the cells' ability to repair DNA damage and maintain viability under stress conditions.

2.2 Objective2: To investigate the influence of STAT3 overexpression on the capacity of hematopoietic stem cells(HSCs) to withstand high doses of radiation.

Rationale: The objective is to clarify the impact of STAT3 overexpression on IR resistance of stem cells. We will assess the role of elevated STAT3 levels by comparing the post-radiation survival rates, functional recovery, and DNA damage repair capabilities of HSCs that overexpress STAT3 to those of control stem cells. This comparative analysis will provide insights into the potential protective effects of STAT3 overexpression against radiation-induced stress.

Methods: Cell Transfection

Cell Preparation: Stem cells are cultivated to the logarithmic growth phase, and the cell density is adjusted to an optimal concentration suitable for transfection.

Plasmid Preparation: Plasmids containing sequences for the overexpression of STAT3 are purified to ensure that their concentration and purity meet the requirements for successful transfection.

Transfection Procedure: Liposome Transfection Method: An appropriate amount of plasmid is mixed with transfection reagents to form a transfection complex, which is then added to the stem cell culture medium. After incubation for a designated period, the medium is replaced with fresh medium.

Electroporation Method: Stem cells are mixed with plasmids and placed in an electroporation cuvette. An appropriate electrical pulse is applied to facilitate the entry of plasmids into the cells. Subsequent procedures adhere to the gene expression analysis outlined in Objective 1.

Expected outcome: Hematopoietic stem cells (HSCs) engineered to overexpressSTAT3 are anticipated to exhibit a diminished level of DNA damage subsequent to radiation exposure. It is hypothesized that the survival rate of these STAT3- overexpressing HSCs post-radiation will surpass that of their normal counterparts, indicating a potential enhancement in IR resistance.

3. Conclusion

This manuscript investigates the viability of hematopoietic stem cells (HSCs) subsequent to exposure to high-dose ionizing radiation. The primary aim of the investigation was to assess the influence of signal transducer and activator of transcription 3 (STAT3) on the radio resistance of HSCs, with a particular focus on the modulation of STAT3 protein expression levels. Employing CRISPR-Cas9 gene-editing technology in conjunction with transfection techniques, the researchers generated HSCs with STAT3 knocked out and STAT3 overexpressed to observe the consequences on cellular radiation response. The study ascertained that STAT3 knockout may render cells more susceptible to high-dose radiation-induced cell death, whereas STAT3 overexpression could potentially confer increased radioresistance. These discoveries offer novel perspectives on the mechanisms by which HSCs endure under severe environmental conditions, presenting prospective targets for the development of novel radiological

protection and therapeutic modalities. Notwithstanding the substantial contributions of this study, it is not without its limitations, including the necessity for additional validation to substantiate the broader applicability of the outcomes. Subsequent research might endeavor to elucidate the role of STAT3 in diverse cell types or under varying radiological conditions.

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