The 5 Rs of Radiobiology: A Focus on Cellular Response to Ionizing Radiation. A Bibliographic Study.

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Abstract.The study of radiobiology is crucial in the field of radiology and radiation therapy as it aims to understand the biological effects of ionizing radiation on living organisms. This understanding is essential for ensuring patient safety and optimizing therapeutic outcomes in radiation therapy. The 5 Rs of radiobiology, which are repair, redistribution, reoxygenation, repopulation, and cell rehoming, play a significant role in the cellular response to radiation. To conduct a literature study in order to explore and analyze the 5 R's of radiobiology (Repair, Reassortment, Redistribution, Reoxygenation, Repopulation) in the context of biology. Overall, the study of the 5 Rs of radiobiology provides insights into the mechanisms by which cells respond to ionizing radiation. It contributes to advancements in radiological protection strategies and improves the effectiveness of radiation therapy. Further research and literature reviews in this field are essential for a safer and more efficient approach to the use of ionizing radiation.This article aims to explore each of these 5 Rs, discussing their mechanisms and importance in radiobiology.

1.Introduction

Radiobiology is a fundamental discipline in the field of radiology and radiation therapy. It studies the biological effects of ionizing radiation on living organisms and aims to ensure patient safety in radiological procedures and optimize therapeutic outcomes in radiation therapy.

Ionizing radiation can cause damage to DNA and other cellular components, affecting the survival and function of exposed cells. Therefore, understanding the mechanisms by which cells respond to such damage is crucial for establishing strategies to limit the adverse effects of radiation on humans. The 5 Rs of radiobiology are important for a better understanding of this cellular response. These topics can be clearly seen in Figure 1.

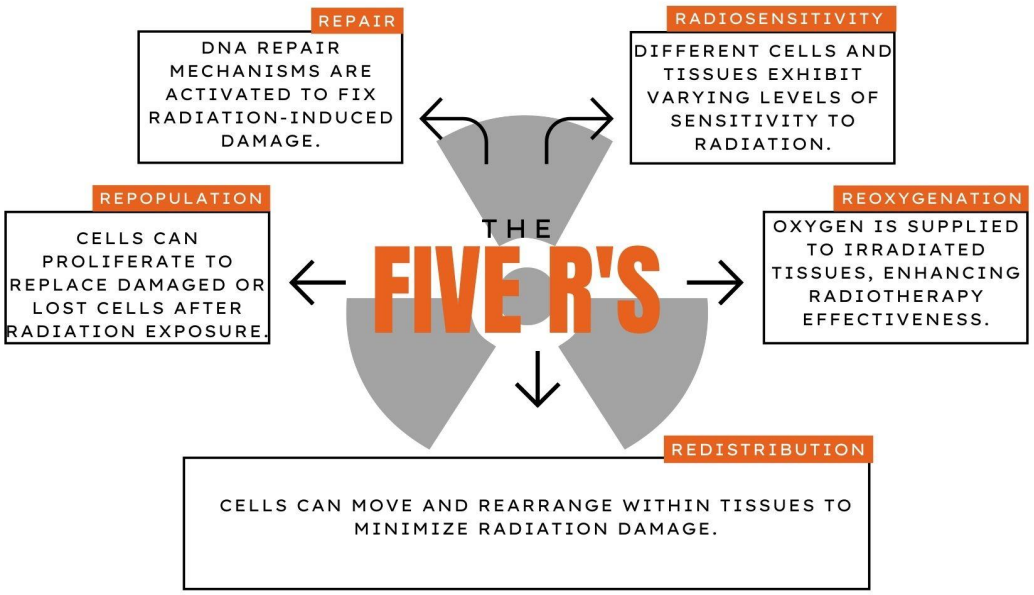

Figure 1: The 5 Rs of radiobiology

Therefore, the study of radiobiology is essential to understand the effects of ionizing radiation and develop strategies that promote greater radiological protection for individuals, whether they are radiation workers or the general public. Thus, understanding the processes established in the 5 Rs contributes to advancements in order to minimize the undesired and adverse effects of ionizing radiation.

1.1 Repair:

This principle refers to the ability of cells to repair the damage caused by radiation. Repair occurs through specific molecular mechanisms that correct DNA damage and restore normal cellular function. DNA repair is a fundamental process for the survival of cells exposed to ionizing radiation, and there are several repair pathways aimed at correcting DNA lesions caused by radiation and maintaining genomic stability.

Nucleotide **E**xcision **R**epair **(NER)** is a mechanism that repairs DNA damage caused by exposure to chemicals, ultraviolet (UV) rays, and other sources of radiation. It acts by removing a sequence of damaged nucleotides and subsequently synthesizing a new sequence of complementary nucleotides to replace it.

On the other hand, **B**ase **E**xcision **R**epair **(BER)** acts on the correction of DNA damage in individual bases, differentiating itself from NER. It removes the damaged base and replaces it with a new complementary base.

Mismatch **R**epair **(MBR)** corrects base-pairing errors during DNA replication, identifying and removing incorrectly paired bases and replacing them with the correct bases.

Another form of repair is **H**omologous **R**ecombination **R**epair **(HRR)**, which is a DNA repair mechanism that occurs during the S phase of the cell cycle, the most important phase for DNA synthesis or duplication. This phase is crucial in the interphase process because it ensures an equal division of chromosomes into daughter cells. It involves the exchange of DNA sequences between homologous chromosomes and is important for repairing double-strand DNA breaks and ensuring genomic stability.
Non-**H**omologous **E**nd **J**oining **(NHEJ)** is a DNA repair mechanism that occurs when there are double-strand DNA breaks. It directly joins the ends of the breaks without the need for homologous sequences, resulting in an imperfect connection.

1.2 Redistribution:
After exposure to radiation, cells may temporarily halt their cell cycle progression, delaying entry into the next phase. This delay is important to allow for DNA repair before the cell proceeds to cell division, thereby avoiding the inheritance of DNA damage.
During cell cycle redistribution in response to ionizing radiation-induced damage, several processes occur that affect the normal progression of the cell cycle. These can be described in 5 main different stages of redistribution that can occur throughout the cell cycle.

a. Cell cycle arrest: After radiation exposure, cells may temporarily halt cell cycle progression at a specific phase. This arrest can occur at any phase of the cell cycle, depending on factors such as the intensity and type of radiation-induced damage.

b. Delay in entry into the next phase: Cells may delay entry into the next phase of the cell cycle after radiation exposure. This allows cells to repair DNA before normal cell cycle processes continue.

c. Activation of cell cycle checkpoint(s): Cell cycle checkpoints are control mechanisms that monitor DNA integrity and proper cell cycle progression. After radiation exposure, these checkpoints may be activated to check for DNA damage and ensure conditions are suitable for cell cycle continuation. Examples of damage include double-strand breaks or damage to nitrogenous bases. When a checkpoint is activated, signaling proteins such as p53, **c**yclin-**d**ependent **k**inases **(CDKs)**, **Ch**eckpoint **k**inase **2 (Chk1)** and **Ch**eckpoint **k**inase **2 (Chk2)** proteins, and **A**taxia **T**elangiectasia **M**utated **(ATM)** and **A**taxia **T**elangiectasia and **R**ad3-related **(ATR)** proteins are recruited to the damaged site, resulting in a cascade of events that lead to cell cycle arrest, allowing for DNA repair. After repair, these proteins are deactivated, and the cell cycle resumes its normal cadence.
It is worth noting at this moment how each of the aforementioned proteins acts in the redistribution process. When we talk about the Chk1 protein, the role of cellular response related to DNA molecule damage can be observed, in which it will assist in the process of genomic integrity, thus preventing cells that have suffered some sort of damage from progressing through the cell cycle. This can be achieved through DNA repair or even apoptosis. On the other hand, the Chk2 protein acts on the regulation of cellular response processes, thus repairing genetic material damage through cell cycle arrest. Severe DNA damage, such as double-strand breaks and other possible damages, are repaired at this stage, and this is done through phosphorylation and activation processes of other proteins that assist in this process. One of the proteins that can be activated at this stage is p53, which in turn

prevents these damaged cells from proliferating. The ATM protein acts in the detection and signaling of DNA damage, activating signaling pathways, and the main one of these repair pathways is called the DNA checkpoint signaling pathway. Another important factor is that this protein can also activate p53, which is essential in tumor suppression processes. Finally, the ATR protein has a similar function to what can be observed in ATM, in which its main function is linked to maintaining the stability of genetic material, thus being able to detect and signal damage.

d. Activation of DNA damage signaling pathways: Cells activate DNA damage signaling pathways, such as the p53 pathway, which triggers a series of events to repair damaged DNA or induce programmed cell death (apoptosis). Other important signaling pathways involved in this process include the ATM/ATR kinase pathway, the BRCA protein pathway, and the RAD9 protein pathway, all working towards genomic and cellular integrity.

e. DNA repair: During cell redistribution, cells can activate DNA repair mechanisms such as nucleotide excision repair (NER) and homologous recombination repair (HRR), mentioned earlier in this article, to correct DNA damage, thereby preventing potential mutations and genetic alterations.

1.3. Reoxygenation:

Reoxygenation refers to the response of irradiated tissue to increase oxygen availability after radiation exposure. Ionizing radiation can lead to relative hypoxia in the tissue due to reduced oxygen supply. However, the presence of oxygen is necessary for radiosensitization. Thus, reoxygenation helps increase the effectiveness of radiation in cancer treatment. This is because radiation can damage blood vessels, reducing the oxygen supply to cells. Among the processes involved in reoxygenation, we can highlight angiogenesis, vasodilation, mobilization of stem cells, increased metabolic rate, and recovery of damaged blood vessels.

Angiogenesis refers to the process of forming new blood vessels from pre-existing vessels in the living organism. After radiation exposure, there is an increase in the production of growth factors and cytokines that stimulate the formation of new blood vessels, which in turn replenish the damaged ones, increasing the oxygen supply to cells.

Vasodilation, on the other hand, functions differently from what was mentioned in the previous topic. Radiation can cause vasoconstriction, narrowing blood vessels and reducing blood flow. As part of the reoxygenation response, vasodilation occurs, resulting in increased blood flow and consequently, oxygen supply to irradiated cells.

When we talk about mobilizing hematopoietic stem cells from the bone marrow into the bloodstream, these cells can differentiate into specialized blood cells such as red blood cells, white blood cells, and platelets. This, in turn, aids in the replenishment of blood cells and consequently increases the transport of oxygen to irradiated tissues. It is worth noting that when we talk about blood oxygenation, this process is carried out by the protein hemoglobin, present in red blood cells. This protein can be divided into types A, A2, F, Gower 1, Gower 2, and Portland, with the first two present only in adulthood, F present both in adulthood in reduced quantities and in the fetal period, and the others in the embryonic development phase. Additionally, an increase in cellular metabolic rate is another factor

that occurs during reoxygenation. This is used to compensate for the decrease in oxygen, thereby increasing efficiency even with limited oxygen availability, which is referred to as hypoxia. Finally, in the process of reoxygenation, we should mention the recovery of damaged blood vessels, which involves the proliferation and migration of endothelial cells, flattened cells that line the interior of blood vessels, to repair the damage in blood vessels, thus improving blood flow and oxygen transport. It is important to note that reoxygenation is crucial for tissue recovery and improving the effectiveness of treatments such as radiation therapy, which rely on oxygen for the production of oxygen free radicals that damage tumor cells.

1.4. Radiosensitivity

Numerous factors can influence cellular radiosensitivity, with particular emphasis on cell type, cell proliferation rate, DNA repair capacity, and the presence of protective mechanisms against radiation damage.

Cells of the hematopoietic and reproductive systems, for example, have a high proliferation rate, making them more radiosensitive compared to other cell types. In contrast, tissues such as bone, muscle, and nerve tissue exhibit lower radiosensitivity, which is directly related to their lower proliferation rate and higher DNA repair capacity. Protective mechanisms against radiation damage also play a significant role in determining an organism's radiosensitivity.

1.5 Repopulation:

Repopulation refers to the process of cell proliferation with a restorative function. However, repopulation can also be a contributing factor to tumor recurrence, due to factors such as selection of resistant cells, stimulation of cell proliferation, favorable tumor microenvironment, and even DNA repair that the tumor cell itself can perform. Biological factors mentioned earlier such as cell migration, angiogenesis, and compensatory cell proliferation are also observed during this time, but we will mainly focus on the activation of quiescent cells.

Some cells may be in a quiescent state, meaning they are at rest, before exposure to radiation. Radiation can activate these cells, causing them to enter the cell cycle and multiply to repopulate the damaged cells.

The activation of quiescent cells is a complex process that involves the reactivation of cellular signaling pathways and entry into the cell cycle. One of the main factors involved in activation is mitogenic signaling, which activates intracellular signaling pathways such as MAPK and PI3K/AKT, promoting entry into the cell cycle.

1.6 Cell Rehoming:

In addition to the 5 r's of radiobiology, the Cell Rehoming is another important point. The movement of normal and damaged cells to new positions within a tissue, allowing surviving cells to fill the empty spaces left by damaged or dead cells. This contributes to tissue recovery and repair, as this cellular reorganization is an adaptive response that minimizes damage and restores normal tissue function. The angiogenesis described earlier also applies to the process of cell rehoming, but other processes such as cell migration are also present in this important moment of radiobiology.

In the process of cell migration, cells can actively move to less damaged or empty areas within the tissue, repopulating and reorganizing the tissue.
Additionally, processes such as compensatory cell proliferation, which replenishes lost cells, cell differentiation, which can occur as part of the rehoming process, restoring tissue functionality, and stem cell activation are some of the processes that can occur after exposure to ionizing radiation. This cellular response and rehoming mechanisms can vary according to the tissue and level of exposure.
Factors such as nutrient availability and epigenetic regulation, such as modifications in chromatin structure that allow gene expression, also play a role in the activation of quiescent cells.
In biological contexts, we observe major events such as tissue regeneration, immune response, and wound healing. However, quiescence can also be associated with pathological conditions as mentioned above, with important implications for the development of therapeutic strategies, both to promote tissue regeneration and inhibit tumor growth.

2. Objective:
The objective is to conduct a comprehensive and in-depth literature study, focusing on the exploration and analysis of the five fundamental aspects of radiobiology, which are Repair, Reassortment, Redistribution, Reoxygenation, and Repopulation. This study aims to investigate and examine these significant components within the context of biology, aiming to enhance our understanding of the intricate relationship between radiation and biological systems.

3. Materials and Methods:

3.1 Selection of databases:
Perform a comprehensive search in major scientific databases such as PubMed, Scopus, and Web of Science, using relevant keywords: "radiobiology", "5 R's", "radiation therapy", "repair", "reassortment", "redistribution", "reoxygenation", and "repopulation".

3.2 Inclusion and exclusion criteria:
Didactic materials and articles focusing on radiobiology, as well as cellular biology, were used for correlation purposes, utilizing works from the year 2000 onwards.

3.3 Selection process:
Filter relevant articles and books based on titles, abstracts, and keywords, and then conduct a thorough reading of the selected articles to assess their suitability for the study's objective.

3.4 Analysis of selected studies:
Extract relevant data from the selected articles, critically analyzing the studies, identifying their limitations, and contributions to the understanding of the 5 R's of radiobiology.

3.5 Organization and synthesis of results:
Conduct a concise synthesis of the main findings from each study, highlighting the importance of the 5 R's.

4. Results:

The bibliographic study of the 5 R's of radiobiology with a focus on biology results in the understanding of the key mechanisms involved in cellular response to ionizing radiation. The results of this literature review indicate that each of the 5 R's plays a crucial role in the cellular and molecular response to radiation. Processes such as repair become essential for cell survival and DNA repair, avoiding genomics-related issues such as mutations. Redistribution and reoxygenation may influence radiosensitivity, making cells more susceptible or resistant to the damage caused by radiation. Cell repopulation relates to the potential for cell growth, while radiosensitivity is an intrinsic characteristic that varies from cell to cell, determining the response it will have when exposed to ionizing radiation.

5. Conclusion:

In radiobiology, we study the effects of ionizing radiation on cells and tissues. Exposure to radiation can cause DNA damage, alterations in the cell cycle, and trigger complex cellular responses.
Understanding the processes of radiobiology is essential for the development of effective therapeutic strategies in radiation biology. Paralell, understanding the effects of ionizing radiation on living tissues justifies and improves the concepts of radioprotection for patients, the public and health professionals.Continuous study and literature reviews ensure a safer and more efficient approach to the use of ionizing radiation. In this way, this study aims to provide a future perspective to promote new advancements in radiobiology, keeping in mind a new possibility for a new R in radiobiology, thus minimizing the effects and doses caused by ionizing radiation more and more.

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