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CURRENT TOPIC / АКТУЕЛНА ТЕМА

Current aspects of radiobiology in modern radiotherapy – our clinical experience

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SUMMARY
Personalized radiation treatment is an important goal in radiation oncology. As a result, one of the main challenges in radiobiology today is predicting a patient's tissue radiosensitivity so that a personalized treatment can be tailored to that individual. For the first time since 2016, a group from the Institute of Oncology and Radiology of Serbia has begun performing translational research in the field of radiobiology. The aim of these studies is to identify molecular markers important for the prediction of radiosensitivity as well as the occurrence of toxic effects of radiotherapy.
In the past five years, significant international cooperation has been established with the Radiogenomic Consortium, as well as leading European and world experts in this field. In addition, during this period, several significant and promising results in this field were published, and a Laboratory for Radiobiology was established at the Institute of Oncology and Radiology of Serbia.
Keywords: radiosensitivity; radiotoxicity; Radiogenomics Consortium

INTRODUCTION

Radiotherapy (RT) is one of the most effective and important non-surgical modalities for the curative treatment of cancer. Unfortunately, RT is not specific only to cancer cells, and radiation-induced cytotoxic effects also occur in normal tissues. In fact, although RT is a local treatment, radiation toxicity can occur both in the RT field area as well as in the surrounding tissues and lead to acute and late complications. It is estimated that a total of 5–10% of patients will eventually develop severe long-term complications that negatively affect quality of life [1].

With improved cure rates, the quality-of-life issue has become increasingly significant, so strategies aimed at reducing toxicity are very important, and to that end, a new field of medicine, called radiobiology, was developed. Radiobiology is a branch of medical science that studies the relationship between the prescribed radiation dose and the consequent radiobiological effects on human cells [2]. Improvements in RT technologies have focused on enhancing precision and accuracy over the years, and as a result, advanced RT techniques such as intensity modulated RT and volumetric modulated arc therapy have been developed [3].

Despite these advances, normal tissue damage remains a limiting factor for RT efficacy, and RT is likely to become much more personalized in the future based on radiobiological factors. The results of researchers in radiobiology, such as the discovery of stem cells and the definition of radiation-activated signaling pathways, represent a fundamental discovery responsible for the further development of radiobiology as well as RT [4, 5].

The functional consequences of ionizing radiation on cells in the broadest sense include cell death, cell repair, effects on the cell cycle, altered gene expression, modification of signal transduction, mutagenesis, and genomic instability [6] (Figure 1).

Nowadays, there is a growing interest in identifying genetic variants associated with an increased risk of radiotoxicity. The reason for this is that it is considered that up to 80% of individual differences in the toxicity of normal tissue caused by RT are conditioned by numerous genetic and epigenetic factors. Many studies of gene expression profiles in peripheral blood lymphocytes of RT-treated patients have indicated an association between certain differentially expressed genes and acute and late radiotoxicity [7]. Therefore, determining the expression profile of a large number of genes by the method of miRNA markers' expression analysis or sequencing of new generation RNA is one of the most important research approaches in modern radiobiology, which allows the discovery of new potential biomarkers of RT effects [7, 8, 9].

Methodological approaches used in cellular radiosensitivity testing include assays to determine cell proliferation and survival, cytogenetic assays, methods to detect DNA damage, and apoptotic assays. Various *in vitro* assays for determining the radiosensitivity of patients' cells, such as lymphocytes or fibroblasts, are used to predict the possible occurrence of toxic effects

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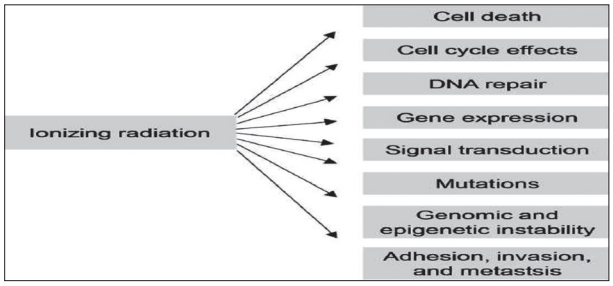

Figure 1. The effect of ionizing radiation on cells [5]

on normal tissue caused by RT. Numerous studies indicate that the level of apoptosis induced by *in vitro* radiation in the peripheral blood lymphocytes of patients before RT is associated with the occurrence of normal tissue radiotoxicity. The existence of a negative correlation between the levels of radiation-induced T lymphocyte apoptosis and the occurrence of toxicity after RT has been shown. Radiation-induced apoptosis of CD4+ T lymphocytes and CD8+ T lymphocytes could be used to identify radiosensitive patients before starting RT [1].

All of these studies should contribute to the identification of a group of patients at an increased risk for developing radiotoxicity. Based on that, an optimal therapeutic approach would be planned for each patient that would enable long-term preservation of the quality of life.

The manuscript was written in accordance with the ethical standards of the institution and the journal.

INITIATION OF RESEARCH AND ESTABLISHMENT OF THE LABORATORY FOR RADIOBIOLOGY

Modern RT uses various strategies and modalities to achieve the most effective clinical effect, and the introduction of individualized RT in clinical practice is imperative. Translational research in RT aims to identify and validate key predictive biomarkers for sensitivity to RT, as well as biomarkers for risk assessment for acute and late toxicity.

Figure 2. Prof. Marina Nikitović and Dr. Tatjana Stanojković at the Radiogenomic Consortium's 2017 annual meeting in Barcelona, Spain

In this way, it will be possible to identify patients prone to radiosensitive reactions before starting RT. This research can help radiation oncologists in the future and is the basis for designing the optimal treatment for the maximum benefit for each patient.

Following the novelty in research, the Institute for Oncology and Radiology of Serbia (IORS) is making constant efforts to strengthen the cooperation between clinicians and researchers, all with the aim of focusing on excellence and the application of research results in practice. In this sense, in 2015, Professor Marina Nikitović, radiation oncologist, and Tatjana Stanojković, Full Research Professor, molecular biologist, launched an initiative to form a team for radiobiology at IORS. Starting from previous studies of acute and late genitourinary toxicity, published in the doctoral thesis of Dr. Vesna Stanković, radiation oncologist, mentored by Professor Marina Nikitović, as well as published papers [10, 11], began the research within the study: "Predictive Significance of Inflammatory Mediators for Early Toxicity and Response to Radiotherapy in Patients with Prostate Cancer" This is the first time in Serbia that new translational research in the field of radiobiology and molecular radiation oncology has been launched at IORS. This investigation was a starting point for the further upgrade of the capacity in radiobiology in Serbia that has been under development for the past five years at IORS. Strengthening these topics, which are in the focus of the most influential European and world centers for cancer research and RT, will make a significant contribution to the advancement of cancer research at a national and international level.

Since 2016, the IORS team for radiobiology consists of researchers and radiation oncologists at the Department of Experimental Oncology, Laboratory for Biological Response Modifiers, as well as the Department of Radiotherapy of Solid Tumors. The team has experience in RT, radiobiology, and molecular RT; the application of enzyme-linked immunosorbent assay for determination of biomarkers; extraction of DNA and RNA from different starting materials; investigating microRNA in cancer; detection of polymorphic variants of various genes (single nucleotide polymorphisms); analysis of specific subpopulations of immune cells in patients by flow cytometry; data processing. Also, transcriptome profiling and radiosensitivity studies for radiotoxicity prediction in patients with prostate cancer have been initiated, as well as epigenetic studies in patients with glioblastoma. As a result of these activities, a Laboratory for Radiobiology was established at IORS.

INTERNATIONAL COOPERATION

From the start, Dr. Tatjana Stanojković and Professor Marina Nikitović, as team leaders, have developed a successful collaboration between the Serbian group and the Radiogenomics Consortium (Figure 2).

Figure 3. Workshop in Mannheim, Germany, within the joint German Research Foundation project (Institute for Oncology and Radiology of Serbia team associates Irina Besu Žižak, Marija Đorđić Crnogorac, Ivana Matić and Željko Žižak), 2017

Figure 4. Team from Serbia, Prof. Marina Nikitović, Dr. Tatjana Stanojković and Dr. Vesna Stanković, in the company of Prof. David Azria and French colleagues from the Radiogenomic Consortium, at the ESTRO meeting 2016 Montpellier France

Figure 5. Prof. Catharine West at the University of Belgrade, Faculty of Medicine, with the Institute for Oncology and Radiology of Serbia team for radiobiology (Prof. Marina Nikitović, Dr. Tatjana Stanojković, Dr. Ivana Matić, Dr. Marija Popović-Vuković, Dr. Dragana Stanić, and Dr. Nina Petrović), and colleagues from the Vinča Institute (Aleksandra Stanković and Ivan Jovanović), 2019

With this cooperation and the started translation research, the IORS is in the company of leading institutes in Europe and the United States in the field of RT and radiation biology (National Institutes of Health, National Cancer Institute, USA, University of Manchester, UK, University of Cambridge, UK, University of Rochester, USA, German Cancer Research Center (DKFZ), Germany). The team for radiobiology has a particularly successful cooperation with the Clinic for Radiation Oncology of the Medical Faculty of Mannheim, the University of Heidelberg, Germany, and the group of Prof. Karsten Herskind. From this cooperation, we especially emphasize the period 2017–2018, when they implemented the project titled "Individual Clinical Radiosensitivity-Toward Predicting and Modulation" under the auspices of the German Research Foundation (project manager on the German side was Prof. Carsten Herskind and on the Serbian side, Dr. Tatjana Stanojković) (Figure 3).

Within the Radiogenomics Consortium, cooperation was established with a research group from the Institute for Cancer Research in Montpellier (l'Institut de Recherche en Cancérologie de Montpellier (IRCM-U1194)) led by Prof. David Azria (Figure 4).

In addition, with Prof. Catharine West, Professor of Radiation Biology, at the University of Manchester, UK, who is also the leader of the European project REQUITE and the founder of the Radiogenomics Consortium, significant cooperation has been established. In 2019, Prof. West gave a notable lecture at the University of Belgrade, Faculty of Medicine, on the topic: "Validating Predictive Models and Biomarkers of Radiotherapy Toxicity to Reduce Side-Effects and Improve Quality of Life in Cancer Survivors – REQUITE project" (Figure 5).

Also, during 2019, within the research in this field, cooperation was initiated with the National Medical Research Center for Radiology of the Ministry of Health of the Russian Federation, Moscow, Russia. The research of the team for Radiobiology of the Institute of Oncology and Radiology of Serbia has also been recognized in Serbia. In 2017, a team of researchers received a grant for research within the "Start for Science" program of the Center for the Promotion of Science.

CONCLUSION

Scientific contribution of the team for radiobiology

Previously, four doctoral dissertations of young radiation oncologists (Dr. Katarina Kopčalić, Dr. Aleksandar Stepanović, Dr. Marija Popović-Vuković, and Dr. Jelena Stanić) were prepared as part of the initiated research, several papers were published in international journals, and several presentations were published at international and national conferences. Currently, a team of researchers has submitted a project proposal within the program

[HORIZON-WIDERA-2021-ACCESS-02-01] – [Twinning Western Balkans special]. The results of the competition are expected in 2022. Further application and participation in national and international scientific projects (Diaspora, Idea, People, Bilateral cooperation, COST actions, EU programs…) is planned (Table 1).

Table 1. Published results in the field of research for 2019–2021

Journal title	Institute for Oncology and Radiology of Serbia publications	Impact factor
Pathology Research and Practice 2019;215(4): 626–31.	Kopcalić K, Petrović N, Stanojković TP, Stanković V, Bukumirić Z, Roganović J, Malisić E, Nikitović M. Association between miR-21/146a/155 level changes and acute genitourinary radiotoxicity in prostate cancer patients: A pilot study.	1.794
Scientific Reports 2020;10(1):19002 Joint publication with University Healthcare Estates & Innovation	Stanojković TP, Matić IZ, Petrović N, Stanković V, Kopčalić K, Besu I, Đorđić Crnogorac M, Mališić E, Mirjačić-Martinović K, Vuletić A, Bukumirić Z, Žižak Ž, Veldwijk M, Herskind C, Nikitović M. Evaluation of cytokine expression and circulating immune cell subsets as potential parameters of acute radiation toxicity in prostate cancer patients.	4.379
Current Medicinal Chemistry, 2021 Aug 3	Petrović N, Stanojković TP, Nikitović M. MicroRNA in Prostate Cancer Radiobiology and Radiotherapy: Towards Prediction of Response to Radiation Treatment.	4.530

Notable published abstracts at international conferences concerning RT and radiosensitivity:

1. Janović Barbara S, Žižak Željko, Vujčić Miroslava T, Vujčić Zoran M, Stanković Vesna, Nikitović Marina, Stanojković Tatjana P. Comet assay as a tool for evaluation of DNA damage in prostate cancer patients after conventionally fractionated 3D conformal radiotherapy 46th EEMGS & 30th GUM Meeting March 18th-21st 2018, Potsdam, Germany.
2. Zizak Z, Janovic B, Besu Zizak I, Vujcic M, Vujcic Z, Stankovic V, Matic I, Nikitovic M, Stanojkovic T. In vitro radiosensitivity and repair kinetics of pbmcs from prostate cancer patients and healthy donors evaluated by comet assay. ESMO Open 2018;3(Suppl 2):A276/PO-129. 25th Biennial Congress of the European Association for Cancer Research, Amsterdam, The Netherlands, 30 June - 3 July 2018. doi: https://doi.org/10.1136/esmoopen-2018-EACR25.653
3. Mališić E, Petrović N, Nikitović M, Stanojković T. Association between TGFB1 C-509T polymorphism and acute toxicity after radiotherapy for prostate cancer. 5th Congress of the "Serbian Association for Cancer Research - SDIR" with international participation „Translational potential of cancer research in Serbia", December 3, 2021 Abstract book p.60. P33.
4. Mališić E, Petrović N, Nikitović M. Impact of TGFB1 Leu10Pro polymorphism on acute radiotherapy-induced toxicity in prostate cancer patients. 5th Congress of the "Serbian Association for Cancer Research - SDIR" with international participation "Translational potential of cancer research in Serbia", December 3, 2021 Abstract book p. 61. P34.
5. Mališić E, Petrović N, Nikitović M. XRCC3 Thr241Met gene polymorphism and acute radiotherapy induced toxicity for prostate cancer. ESTRO 2022. 06-10 May 2022, Copenhagen, Denmark.

Conflict of interests: None declared.

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7. Benitez CM, Knox SJ. Harnessing genome-wide association studies to minimize adverse radiation-induced side effects. Radiat Oncol J. 2020;38(4):226–35. [DOI: 10.3857/roj.2020.00556] [PMID: 33233031]
8. Moreno-Villanueva M, Zhang Y, Feiveson A, Mistretta B, Pan Y, Chatterjee S, et al. Single-Cell RNA-Sequencing Identifies Activation of TP53 and STAT1 Pathways in Human T Lymphocyte Subpopulations in Response to Ex Vivo Radiation Exposure. Int J Mol Sci. 2019;20(9):2316. [DOI: 10.3390/ijms20092316] [PMID: 31083348]
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11. Stankovic V, Nikitovic M, Pekmezovic T, Kisic Tepavcevic D, Saranovic Dj, Djuric-Stefanovic A, Saric M. Toxicity of the lower gastrointestinal tract and its predictive factors after 72Gy conventionaly fractionated 3D conformal radiotherapy of localised prostate cancer. J BUON. 2016;21(5):1224–32. [PMID: 27837627]

Актуелни аспекти радиобиологије у савременој радиотерапији – наше клиничко искуство

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САЖЕТАК

Персонализована радиотерапија је важан циљ у радијационој онкологији. Стога је један од главних изазова у радиобиологији данас предвиђање радиосензитивности нормалног ткива болесника како би се радиотерапијски третман прилагодио сваком болеснику понаособ. Група са Института за онкологију и радиологију Србије први пут је од 2016. године почела да развија транслациона истраживања из области радиобиологије.

Циљ ових студија је идентификовање молекуларних маркера важних за предвиђање радиосензитивности, као и појаву нежељених токсичних ефеката радиотерапије.

У протеклих пет година основана је значајна међународна сарадња са Конзорцијумом за радиогеномику, као и водећим европским и светским стручњацима из области радиогеномике. Такође, у овом периоду објављено је неколико значајних и обећавајућих резултата и основана је Лабораторија за радиобиологију на Институту за онкологију и радиологију Србије.

Кључне речи: радиосензитивност; радиотоксичност; Конзорцијум за радиогеномику