Prospects for Radiopharmaceuticals as Effective and Safe Therapeutics in Oncology and Challenges of Tumor Resistance to Radiotherapy

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Review

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Abstract

The rapid advances in nuclear medicine have resulted in significant advantages for the field of oncology. The focus is on the application of radiopharmaceuticals as therapeuticals. In addition, the latest developments in cell biology (the understanding of the cell structure, function, metabolism, genetics, signaling, transformation) have given a strong scientific boost to radiation oncology. In this regard, the article discusses what is soon going to be a new jump in radiation oncology based on the already accumulated considerable knowledge at the cellular level about the mechanisms of cell transformation and tumor progression, cell response to radiation, cell resistance to apoptosis and radiation and cell radio-sensitivity. The mechanisms of resistance of tumor cells to radiation and the genetically determined individual sensitivity to radiation in patients (which creates the risk of radiation-induced acute and late side effects) are the 2 major challenges to overcome in modern nuclear medicine. The paper focuses on these problems and makes a detailed summary of the significance of the differences in the ionizing properties of radiopharmaceuticals and the principle of their application in radiation oncology that will shed additional light on how to make the anti-cancer radiotherapies more efficient and safe, giving some ideas for optimizations.

Keywords

radiopharmaceuticals, cancer, apoptosis, radio-sensitivity, radio-resistance

Introduction

Radiation oncology aims at selective cytotoxic effects on the tumor cells in the patient's body with the application of ionizing radiation. In this regard, the cytotoxic effects of the anticancer radiotherapy are a complex function of 3 factors—the type of the applied therapeutic ionizing radiation; the patient's cancer cells' radio-resistance; and the genetically determined individual radio-sensitivity of the cancer patient which determines each patient's individual tolerance to therapeutic irradiation and the risk of acute/late side effects (Figure 1).

Despite the considerable advances the nuclear medicine has achieved over the last decades, the latest global cancer data of the World Health Organization reported 9.6 million cancer deaths in 2018 against the background of 18.1 million new cases of cancer patients registered in the same year [https://www.who.int/cancer/PRGlobocanFinal.pdf]. It brings the need for further optimization of the anticancer radiotherapies which requires constant reviews of the numerous research publications in physics, radiobiology and cancer biology. This review points out some options for optimization summarizing the already accumulated significant scientific knowledge on: the

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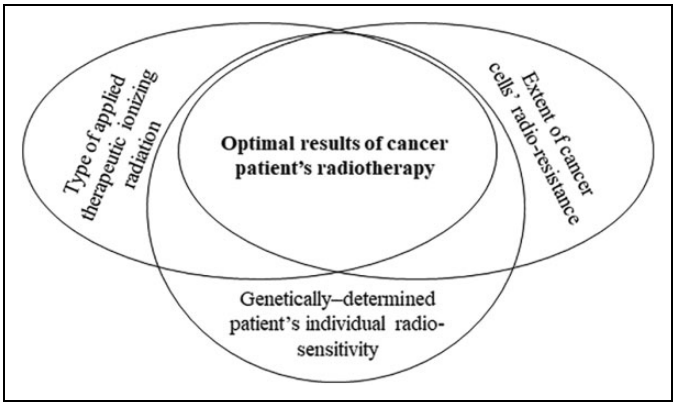

Figure 1. Set of factors determining the optimal results of a cancer patient's radiotherapy.

nature of ionizing radiation; the ionizing effects of radiopharmaceuticals at the cellular level and how these effects differ depending on the nature of the radiopharmaceuticals; the mechanisms of the radio-resistance in tumor cells, and the genetically determined differences among individuals in regard to their radio-sensitivity. Our final goal is to discuss the potential of ionizing radiation to provide an effective antitumor therapy capable of defeating therapy-resistant cancer cells based entirely on the molecular mechanisms of these processes.

Nature and Types of Ionizing Radiation and Their Relation to the Elicited Intracellular Ionizing Effects

The knowledge of the differences in the nature of ionizing radiation resulting in different ionizing effects at the cellular level and the cell's response to radiation can be used to improve the efficiency of anticancer radiotherapy. The cytotoxic anti-cancer effects in radiation oncology are provoked by radioactive isotopes of well-known chemical elements called "radionuclides." However, the term "radionuclides" is very common and includes a huge variety of radionuclides with different physical and chemical properties which reflects the ionizing and cytotoxic effects of each radionuclide, making it unique. The ability to predict and control the biological effects of the radiopharmaceuticals applied in nuclear medicine is only possible if there is an understanding of the physical and chemical characteristics of each radiopharmaceutical. The good knowledge about the nature of ionizing radiation helps in the following directions in the therapeutic applications of radionuclides, allows: to measure and control the radiation dose and dose rate based on the half-life of the specific radionuclide (and its daughter nuclides); to determine the ionizing strength of the radionuclide by the number of the ionizing events caused per unit area (the so-called Linear Energy Transfer (LET) values of the radionuclides); to predict the predominant type of the DNA damage that occurred—base oxidations and single strand-brakes versus more complex double-strand brakes based on the mass and the charge of the ionizing particles; and to predict the specific penetration ranges of the different types of ionizing radiation into matter (respectively into the human body) and to calculate the diameter of the ionizing effect of the targeted radionuclides.[1-4]

The possibility to determine the dose and dose rate of the radiation of a given radionuclide has led to 3 beneficial applications of radiation in medicine: for diagnostic purposes—to label cells; for therapeutic purposes—to induce healing through regeneration; and for cytotoxicity—to kill cells. These could be achieved in different ways.

At a low dose and with a targeting strategy (which in addition requires good knowledge about cell biology), suitable radionuclides with a short half-life can be used to achieve safe and functional cell-labeling and visualization in the multicellular human body for the purpose of the nuclear diagnostic techniques such as PET/CT, SPECT/CT, PET/MRI. The shorter the half-life and metabolism of the radiopharmaceutical, the more suitable for diagnostic purposes. Gamma and positron emitters with low LET values are preferable because of their weaker ionizing properties in comparison to alpha radiopharmaceuticals.

At low doses and at low rates, radiation may provide medicine with another benefit—to induce cell repair and tissue/organ regeneration through the so-called mild stress activated protection system in each living cell.[5-7] Billions of years ago life on our planet appeared and existed in an environment with several orders of magnitude higher levels of natural radiation.[8] Thus, the single cells and the cells of multicellular organisms have developed their complex intrinsic antioxidant defense which performs 2 functions—first, to eliminate the free radicals generated by the radiolysis of the cellular water after irradiation (known as "indirect" oxidizing effects of radiation on cellular biomolecules—proteins, lipids, nucleic acids) and second, to detect and repair the consequences of the "indirect" and also of the "direct" ionizing effects of radiation on the cellular DNA molecules after it deposits its energy directly to eject electrons from these important macromolecules of the cells. In this regard, the cells within the multicellular human body have developed a very complex and multileveled defense system. It includes intracellular antioxidant enzymes (catalase, superoxide dismutase, peroxidase, peroxiredoxins),[6,9] glutathione,[10] free radicals scavenging pigments—intrinsic to the cells (melanin)[11] or food-supplied pigments (phytochemicals),[12] vitamins (A, C, E),[13,14] hormones (melatonin).[15] Cellular lipids and microRNAs are also an important part of the different mechanisms working together to deactivate directly the dangerous free radicals or to protect the cell by activating a strong anti-apoptotic and/or pro-survival intracellular signaling pathways.[16-20] In addition, the cellular antioxidant defense system includes DNA damage detecting and repairing enzymes (over 130 DNA repairing enzymes operate in each human cell in a highly organized manner).[21,22] Another level of the cellular antioxidant defense is represented by the mechanism of the "protective apoptosis" which cleans the irreversibly damaged cells out of the multicellular organism, thus protecting the whole organism against diseases (the

p53-dependent and p-53-independent apoptosis, 2 mechanisms that are obviously lost in every radio-resistant cancer cell).[23,24] This ability of the cells to protect themselves against radiation creates the so-called "Hormesis Theory" in biophysics and allows the use of ionizing radiation at low doses and low rates to achieve regeneration of tissues with chronic inflammation (atopic dermatitis, asthma, rheumatoid arthritis) or for curing people with cancer.[25-27]

At high doses and high dose rates, ionizing radiation becomes cytotoxic and this is used in radiation oncology to target and kill tumor cells. Since in oncology the dose and dose rate can not be increased unrestrictedly in achieving an efficient cytotoxic antitumor effect due to the radiation tolerance of the normal organs and tissues surrounding the tumor,[28] other characteristics of the radiopharmaceuticals are to be considered for their efficient cytotoxicity. Research experiments from the past years demonstrate that the higher the LET of the radionuclide, the bigger the complexity and the number of the DNA double-strand breaks versus the DNA single-strand breaks.[1,4] The bigger the complexity of the DNA damage, the higher the effectiveness against even the strongest anti-apoptotic mechanisms in all radio-resistant tumor cells as such cytotoxicity is able to cause death in tumor cells through necrosis rather than apoptosis.[29] In biophysics the high LET values are demonstrated by α-particle-emitting radionuclides followed by beta and gamma radionuclides. How does this change the biological effectiveness of radionuclides to such an extent that research and clinical data are replete with examples of beta- and gamma-resistant tumor cells even at high radiation doses but at the same time there are no examples of alpha radiation-resistant tumor cells? Since the specific physical characteristics of the different types of ionizing radiation are critical for the final results of the application of radiopharmaceuticals in radiation oncology, a brief discussion in the following 3 paragraphs is needed on why the different nature of radiation elicits differences in the number and complexity of the DNA strand breaks, the extent of the observed gammaH2AX-foci formation, the induction of cell cycle checkpoint and DNA repair instead of cell apoptosis or necrosis.

According to its nature ionizing radiation is an emission (or transmission) of energy that comes from the radioactive decay of an unstable radioisotope (a radionuclide) and in some cases this energy is in the form of highly energetic electromagnetic waves (photons), in other cases it is highly energetic subatomic particles (protons, electrons, bare helium atoms) that are able to eject electrons when hitting an atom of a molecule causing ionization of the molecule (which is the principle of radiation cytotoxicity in the context of radiation application as an anti-tumor treatment).

Depending on the nature and size of the ionizing particles, the ionizing effects and the penetrating power of the different types of ionizing radiation differ. The largest and heaviest alpha particles, compare to gamma and beta ones, penetrate the least (μm into a matter such as the human body) and since they are doubly charged they are relatively slow and show bigger ionizing effects (the strongest cytotoxic effects) into matter than the photons which have zero electrical charge and zero mass. Having no mass or charge, gamma radiation can travel much farther through air or matter than alpha or beta radiation and thus has greater penetrating ability (measured in cm into the human body). Beta particles in contrast to photons possess a charge and a mass, but in comparison to alpha particles they have a single charge (positive or negative) and a 8000 times smaller size. Thus, beta particles interact less strongly with matter than alpha particles, traversing through the human body with penetration ranges of mm to a few cm. It means that in regard to its ionizing properties beta radiation ranks between gamma and alpha radiation.

It also gives us an explanation of why the different nature of ionizing radiation results in a different outcome at the cellular level which reflects the strength of the cytotoxic effects of the various radiopharmaceuticals at the same doses and dose rates applied for treatment in radiation oncology. The successful results in radiation oncology depend on the right choice of a radionuclide which in turns depends on the final goal—diagnostics or treatment.[30] While the medical diagnostic techniques using radionuclides in the form of tracers excelled and achieved great precision due to the rapid advances in cell biology, immunology, physics, pharmacochemistry, medicine, and engineering with the introduction of PET/CT- and SPECT/CT-scanners for positron- and gamma-emitting radiopharmaceuticals, there is still a long way to go until the full potential of radionuclides as successful anti-cancer therapeutics is reached. This is especially true for alpha radionuclides which until recently have been considered of no medical use because of their strong ionizing properties.[27,30,31]

The next part of the review considers already available scientific evidence that with the right scientific strategy alpha-radionuclides can not only find their medical application but also save lives where the weaker ionizing photon and beta radiations fail in the treatment of apoptosis-resistant tumors and even speed up the tumor growth with their weaker ionizing properties.[27,28,32]

Tumor Cells Radioresistance as a Function of Complex Factors—a Large Number of Hidden Pro-Survivalmechanisms in Tumor Cells, Limited Diagnostic Capabilities in Clinics and Wrong Approaches in the Application of Antitumor Strategies

Cancer has become a scourge for modern society since the factors provoking it have multiplied in everyday life—stress, poor diet, pollution, artificial toxic substances in food, the aging population of the developed countries, some inherited genetic factors, etc. The lack of response to the existing chemo- and radio-therapies in almost 50% of cancer patients forces scientists to continue studying the resistance mechanisms of tumor cells.[18,19,20,28,30,33-38] With no intention to be exhaustive, Figure 2 summarizes schematically the diversity of these mechanisms. The need to search for stronger cytotoxic

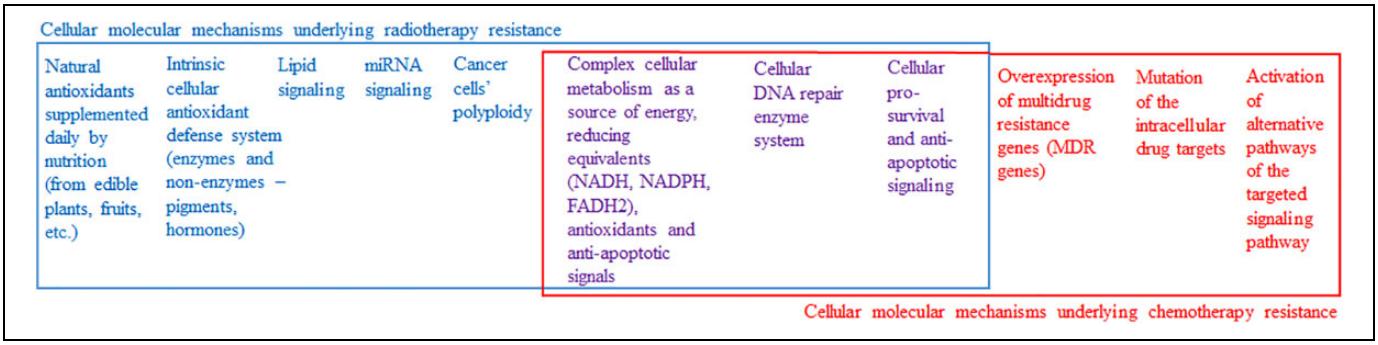

Figure 2. Complexity of the pro-survival mechanisms of cancer cells under cytotoxic conditions. The mechanisms of cytotoxicity of anti-tumor radiotherapy and chemotherapy are different. Accordingly, the mechanisms used by tumor cells to counteract this toxicity also differ markedly. The specific cellular molecular mechanisms underlying the radio- and chemo-therapy resistance are presented in blue and red respectively. Where radiotherapy and chemotherapy are intended to achieve genotoxicity through oxidation and DNA strand breaks, tumor cells use the same counteracting mechanisms such as activation of strong anti-apoptotic signaling pathways as well as activation of DNA-repairing pathways (the cellular mechanisms indicated in purple).

therapies that could be targeted and are capable of defeating this cancer cells' resistance, whatever its molecular mechanisms are, is becoming more and more apparent. The extended cancer research has revealed that cancer is a disease connected to normal cell transformation due to randomly acquired one or more mutations resulting in a dramatic change of the otherwise well-controlled intracellular signaling pathways for cell growth, capable to reverse cell differentiation, to suppress apoptosis and, unfortunately, to assure long-term survival under cytotoxic conditions exceeding the survival rate of normal cells. The more research and clinical data accumulate, the more obvious it becomes how easily some tumor cells are able to overcome the cytotoxic effects of photon- and beta-emitters which are the predominating radio-therapeutics in the current clinical practice because of their weaker ionizing properties in comparison to alpha radiopharmaceuticals.[30,31] For example, MOLT-4 human T lymphoblasts, isolated from a patient with an acute lymphoblastic leukemia and irradiated with a 100 Gy proton beam, 24 h after the irradiation show no signs of induced apoptosis. Other cells famous for their resistance to treatment such as Head and Neck Squamous Cell Carcinoma (HNSCC) cells, also survive after repeated exposure to X-rays ionizing radiation with a total dose of 100 Gy and researchers are amazed to observe that the ionizing-resistant cells retained their radiation resistance even after 3 years of *in vitro* passaging.[32,37] These doses of beta and photon radiation exceed the tolerance of normal tissues and organs to radiation and are still not effective against tumor cells.[28]

The advance in radiation biology today, especially after the discovery of radiation biodosimetry, has revealed that the problem with cancer cell resistance to radiotherapy is multifaceted. On the one hand, the photons and beta particles cannot induce efficient mitochondrial and/or DNA damage capable of triggering cell apoptosis, mitotic catastrophe or necrosis.[1,29,32,39] On the other hand, the mechanism of the cytotoxic effect of photon and beta radiation (which includes activation of apoptosis as a secondary effect of the radiation-induced free radicals generation)[4,39,40] may be counteracted by many intracellular mechanisms in apoptosis-resistant tumor cells (Figure 2). These mechanisms for counteraction include—efficient neutralization of the generated free radicals[41-43]; activation of prolonged check points for DNA-strand breaks repair[36]; constitutive activation of strong pro-survival and anti-apoptotic signaling[37,44] or impaired activation of pro-apoptotic signaling.[45] The complexity of the pro-survival mechanisms of cancer cells under cytotoxic conditions grows with the observation that these mechanisms involve not only protein signaling but also lipid-[18] and miRNA-signaling.[20,46] The current chemotherapies usually target deregulated protein signaling in tumor cells such as growth factor receptors or another downstream of the receptor oncogenes in an attempt to sensitize them to the concomitant radiotherapy[28,33] but at the same time the lipid- and miRNA-signaling that function in parallel to protein signaling to support the radio-resistance of tumor cells is not considered during therapy. Undoubtedly, this will complicate significantly and increase the cost of the preceding diagnostic process, which will have to identify the lipid- and miRNA-signaling involved in tumor cells' resistance to therapy in parallel to protein tumor markers.

Another factor that can contribute to the complexity of cancer cells resistance to therapy and compromise the efficiency of anti-cancer radiotherapy is cancer cells polyploidy.[47,48] According to the cited studies, for example, a significant percentage of all breast and pancreatic cancers are polyploid. The polyploidy is a predictor of cancer recurrence or death, the authors say. Cancer cell polyploidy can be considered a predictor of radiotherapy resistance too. More chromosomes means more copies of genes coding for enzymes of the antioxidant defense system and more genes for DNA repair proteins which will weaken the cytotoxic effects of the radiation in the irradiated tumor cells. However, when determining the efficient therapeutic radiation dose software such as Geant4 misses to consider in its calculating algorithms that polyploid cancer cells survive better than diploid cells after irradiation under the attack of the same number of radiation-generated genotoxic free radicals.[4] The analogy of polyploid tumor cells with the radioresistance record-holder—the

bacterium *Deinococcus radiodurans*, which is distinguished by the large number of copies of its genome, is self-imposed.[49]

The diet of cancer patients during radiotherapy can also affect the efficiency of the radiotherapy in many ways which calls for some discussion on this point. The fact that radiation can cause cancer[50] and at the same time can cure cancer requires extremely detailed knowledge of the mechanisms of radiation action at the cellular level and the mechanisms by which cells counteract these effects. Radiation is part of our daily life since the emergence of life on earth[8] and the living cells have developed mechanisms to sense it[51,52] and to protect themselves from it.[53] Our food is important part of these mechanisms. A diet rich in fruits, vegetables and herbs is chemopreventive (protective against oxidative damage) for healthy individuals due to the potent antioxidant activity of its vitamins (carotenoids, tocopherols, ascorbic acid), minerals (Cr, Mn, Se or Zn), phytochemical compounds (polyphenols, alkaloids, saponins, glycosides, resins, oleoresins, sesquiterpene, and lactones) which leads to 1) reduced oxidative damage in macromolecules such as DNA and lipids by scavenging the reactive oxygen, nitrogen species and chelating redox-active transition metal ions, 2) inhibition of both the apoptotic process induced by ROS and the redox-sensitive transcription factors; 3) inhibition of "pro-oxidant" enzymes, such as inducible nitric oxide synthase, lipoxygenases, cyclooxygenases and xanthine oxidase; 4) induction of antioxidant enzymes, such as glutathione-S-transferases and superoxide dismutases 5) positive modulation of radiation-induced DNA damage repair.[12,13,19,38,54,55] For healthy individuals or individuals undergoing radio-diagnostic procedures this food antioxidant activity is beneficial but during anti-cancer photon and beta radiotherapy it can attenuate the cytotoxic effects of the irradiation on tumor cells. In this regard, despite being debatable and largely rejected in some standard oncology practices, the therapeutic effects of short-term fasting during anticancer therapy should be discussed.[56-59] Clinical studies confirmed that when conducted exclusively under medical supervision and after careful assessment of the patient's condition (early-stage cancer with no signs of severe weight loss, diabetes, sarcopenia, cachexia or eating disorders), short-term water-only fasting just before and after radiotherapy could enhance the cytotoxic effects on the tumor cells at many levels.[56] Such an additional metabolic therapy contributes to the increased stress on oncogene-driven tumor cells during chemo/radio therapy—it reduces the amount of their main source of energy and building blocks for growth, DNA repair and poliferation, gradually weakening all anti-apoptotic signaling pathways maintained by nutrition. Fasting is used as a strategy to lower the serum levels of glucose, insulin and insulin-like growth factor 1 (IGF-1) just before, during and after therapy which is critical for the tumor cells survival as glucose fuels tumor cells with energy in the form of ATP through both aerobic or anaerobic (hypoxic tumor growth) glycolysis. Insulin and IGF-1, in their turn, activate the cellular pro-proliferative Ras/MAPK signaling pathway and the strong anti-apoptotic PI3K/AKT pathway.

When antitumor radiotherapy is not successful, the reasons usually are sought in the failure of the DNA damage-induced p-53-mediated pro-apoptotic signaling but the lack of activation of the cytoplasmic mitochondrial apoptotic pathway is not discussed. Fasting is the simplest and non-toxic strategy to sensitize tumor cells to radiation-induced apoptosis through switching off the "insulin→Akt→Hexokinase II→mitochondrial protection→cell survival pathway" in them, a signaling already observed to be protective against oxidative injury in adenocarcinomic human alveolar basal epithelial cells A549 for example.[60] The active AKT is cental in mitochondrial protection and intracellular antiapoptotic mechanisms, it inhibits also the Ca^{2+}-induced pathway for apoptosis, transcriptionally up-regulates the anti-apoptotic Bcl, and inhibits through phosphorylation the pro-apoptotic Bax and Bad. Any chemical inhibition of Akt protein as an antitumor chemotherapy can be risky since this protein is critical for the viability of the cardiac muscle cells (the so called chemotherapy-induced cardiotoxicity).[60] Fasting can weaken naturally Akt-signaling in oncogene-driven tumor cells facilitating their apoptosis when in healthy cells oncogenes switch off and only re-investment of energy in maintenance and repair is induced. This well-observed distinct response of healthy versus tumor cells to fasting is called "differential stress resistance" (DSR). It protects all healthy cells from the detrimental effects of chemo- and radio-therapy observed in the form of severe acute and late side effects at a body level (fatigue, nausea, vomiting, cachexia, inflammation, secondary cancer). More importantly, the DSR phenomenon promotes efficient anti-tumor immunity in the human body through mechanisms discussed by Groot et al. in their review.[56] Instead, the standard medical protocols in the clinics include immunosuppressive corticosteroids to suppress the side effects of chemo- and radio-therapy. Fasting can also facilitate the p53-induced apoptosis through Akt-inhibition or even compensate for the lack of p53-mediated Akt-inhibition in tumor cells.[61]

In the context of anti-tumor radiotherapy, fasting is beneficial in yet another way—it deprives temporarily tumor cells of fats. Fats are a great source of energy (fat provides 9 calories per gram whereas carbohydrates provide 4 kcal per gram and protein—4 calories per gram) but at the same time, dietary fats supply tumor cells with important structural materials for building their membrane structures necessary for growth and proliferation, contributing to faster recovery of tumor cells after irradiation. Last but not least—fat metabolism supports the synthesis of cofactors such as lipoic acid for enzyme function and one of the strongest known antioxidants inside the cells.[16] As Ralph et al. point out in their review[62] cancer cells show significantly increased levels and activity of the enzymes involved in metabolizing fatty acids both for synthesis and degradation of these energy sources.

Currently, the cytotoxic oxidative effects on tumor cells pursued in clinics by photon and beta antitumor radiotherapy are largely failed and we argue that this is partially a result of lack of participation in the treatment team of specialists in tumor signaling and metabolism (extremely extensive areas in tumor biology) and a certified dietitian. This type of

professional intervention is critical because even the positive effects of fasting during antitumor radiotherapy may be hampered by factors as obesity due to the excess fat stored in adipose tissue,[63] and nutrient deprivation–induced autophagy in tumor cells conferring their survival through the signaling pathway: nutrient deficiency→energy shortage→increased AMP/ATP ratio→AMPK activation→mTORC1 inhibition→autophagy induction for energy production.[29,44,64-67] Not only nutrient deprivation but also activational mutation in autophagy related proteins or radiation in itself through LKB1-phosphorylation and activation is able to induce the same signaling pathway for autophagy which should be considered a possible mechanism of tumor cells survival during radiotherapy.[52,64] Despite all this knowledge, it is not yet a concomitant part of the clinical practices of antitumor photon and beta therapies and as such it can not help in increasing the effectiveness of these therapies.

One of the many reasons for the lack of induced apoptosis in tumor cells after radiotherapy is the defective signaling proteins controlling positively the activation of the protease and nuclease enzymes performing the process of programmed cell death. An inactive p53-protein is often observed in cancer patients not responding to therapy and strategies for restoring its function have been suggested as anti-cancer therapies.[68] However, p53 is just a single part of a very complicated nuclear signaling network of more than 130 DNA-checking and repairing proteins which work in synchrony to keep the integrity of the cellular DNA, to check for DNA base oxidations, to detect single- and double-strand brakes, to accomplish the right mechanism for DNA repair and, in case of unrepairable DNA damage, to signal for activation of the process of protective apoptosis in the cells with defective DNA which is critical to the health of multicellular organisms. The above said should be considered when planning a chemotherapy accompanying the radiotherapy with inhibition of the DNA repairing proteins—a strategy already applied in clinics[28] or often discussed in scientific reviews,[69] since in this way pro-apoptotic signaling can be inhibited in tumor cells, for example PARP-mediated or DNA-PK-/ceramide-mediated apoptosis induction.[40,70-74]

Since gamma- and beta-emitting radionuclides are applied in the clinic as antitumor therapeutics to induce apoptosis in tumor cells as a secondary effect of irradiation and free radicals generation,[29,32] all possible reasons for the lack of success of these therapies have to be discussed regardless of how numerous they might be. The radiotherapies that generate free radicals and DNA-base oxidations in tumor cells but are not sufficient to induce apoptosis can be dangerous rather than helpful for the cancer patients. The reason is that if tumor cells possess active mechanisms to efficiently decrease the cytotoxicity of free radicals, then at moderate concentations free radicals have proven able to exert physiological control on important cellular functions, for example, to stimulate cell proliferation and motility,[75,76] to induce prolonged cell cycle checkpoint for DNA repair,[36,44] to generate more gene mutations that lead to more mutated proteins not responding to the chemical inhibitors of the ongoing chemotherapy, the so-called "acquired chemotherapy resistance."[77,78]

In almost 50% of the cancer patients treated with the current photon or beta radiation techniques in the clinic the tumor cells do not respond in the expected way with induced apoptosis although these cells possess all the physiological features of cells that should be very sensitive to radiation. They have a faster metabolism than normal cells for energy production which charges them with more reactive oxygen species—superoxide radicals during the process of ATP synthesis and hydrogen peroxide from the fatty acids oxidation.[62] A tumor cell contains more water than a normal cell[79] which is supposed to make it more vulnerable to radiation. Tumor cells proliferate abnormally[80] which should make them more sensitive to cytotoxic effects as it happens with all normal quickly dividing stem cells in the human body or with embryonic cells after irradiation.[81,82] In addition, during radiotherapy the radiation targeting the tumor cells generates free radicals in these cells not only by water molecules radiolysis but also through direct activation of derivative systems such as NADPH oxidase and inducible NO synthase (iNOS).[83] Instead of becoming apoptotic under all these conditions the therapy resistant tumor cells survive and even happen to accelerate their growth after irradiation.[27,28,30] It is obvious that the current clinical practice needs stronger cytotoxic anti-tumor therapy capable to defeat the complex anti-apoptotic strength of the tumor cells resistant to therapy. Nuclear physics and nuclear medicine can offer such a therapy through the common introduction of alpha-radiopharmaceuticals as antitumor therapeutics in the clinical practice.

The way of alpha-radionuclides to the clinic has already been paved by convincing clinical data confirming the risk of the application of an internal targeted beta-radiotherapeutical (^{177}Lutetium-PSMA-617 ligand) in the treatment of a patient with an advanced apoptosis-resistant metastatic prostate cancer resulting in increased values of the tumor marker after the beta-radiotherapy and, on the other hand, the life-saving results after the application of an internal targeted alpha-radiopharmaceutical (^{225}Actinium-PSMA-617ligand) in the same patient.[27] The efficient anti-tumor cytotoxicity and the safe range of penetration of the alpha-emitter for the patient with tumor cells resistant to beta-radiation and with red marrow metastasis are obvious. As a well-designed targeted immunotherapy and with proper chelators assuring selective targeting to cancer cells and strong binding with a quick release out of the body of the radionuclide respectively, the targeted alpha-radiotherapy is the long-waited solution for patients with difficult-to-treat tumor cells.[27] The reported lack of serious acute side effects during alpha radiotherapy with ^{225}Actinium is also very encouraging.

The diversity in the chemical properties of the different existing alpha-emitters creates different treatment options for cancer patients, for example by an internal non-targeted inhalation therapy with the noble gas ^{222}Radon (^{222}Rn). The radionuclide ^{222}Rn combines the properties of a chemically inert noble gas and alpha-emitter with a relatively short half-life

(3,8d); its pharmacokinetics shows that when it is inhaled or absorbed through the skin, radon is released out of the human body in 50-60 minutes, a period much shorter than its half--life.[84] The radon inertness and its quick release out of the body explain why radon as internal nontargeted radiotherapy is capable of causing just mild injuries in the human body but enough to induce regeneration without any serious harm to the organism. Such an anti-cancer therapy continues a bit longer, from 8 to 12 months, but has proven effective in curing 2 advanced breast cancer patients with numerous metastasis including brain metastasis proven by 3 years of complete remission.[27]

Inter-Individual Differences in Human Radiosensitivity

Like all other medicines, radiopharmaceuticals should also be provided with detailed instructions about any contraindications. Biodosimetry based on gammaH2AX foci assay has to become an integral part of all radiation procedures in the clinic both diagnostic and therapeutic.

In vitro biodosimetric tests with peripheral blood cells (lymphocytes) of healthy volunteers have provided data that improved the understanding about human sensitivity to genotoxicity which may assure the safe application of all radiopharmaceuticals in the clinic. Two risky groups of people have been identified with regard to the biodosimetric technique. The first group of people is characterized by the observation that upon genotoxic treatment with the same dose as other people a greater initial number of DNA gmmaH2AX foci are formed in the nuclei of their cells and this phenomenon is not related to gender or age.[85] It has been suggested that the observed higher initial number of DNA breaks can be associated with the unlocking of more acute adverse reactions (neutropenia, febrile neutropenia, thrombocytopenia, gastric or duodenal ulcer, ascites) during chemo- and radio-therapy with these patients. Short-term therapeutic fasting 24-48 hours before therapy could accompany chemo- and radio-therapy in such cases as it has been proven clinically to help reduce the side effects of cytotoxic therapies.[56,64] It should also be checked whether radon therapy for such patients is a more appropriate option as it induces slow regeneration during a longer period of treatment with low doses and low dose rates instead of using strong cytotoxic high radiation doses and high dose rates.[26,27]

As already mentioned, a second group of radiosensitive people has also been identified by means of biodosimetry. In this group of people the radiation-induced DNA foci demonstrate significantly delayed kinetics in their repair. Since DNA foci have strict kinetics of formation and repair,[86,87] any delay in their repair time indicates some DNA repair enzyme deficiency which leads to a greater residual number of unrepaired DNA foci 24 hours after irradiation.[88,89] For these people any form of radiation is contraindicative and carcinogenics[90] and other diagnostic and anti-tumor treatment options must be considered.

The study of the molecular mechanisms of the genetically-determined radiosensitivity in people must continue with the techniques available today in modern radiobiology and biodosimetry.[91] The identification of radiosensitive people is of fundamental importance because if they stay unidentified and when admitted as patients in radiation oncology clinics, there will always be a compromise on the positive results from the application of radiation and radiopharmaceuticals as a therapeutic treatment.

Conclusions and Recommendations

The already accumulated large amount of scientific data makes the introduction of functional tests for radiosensitivity in the clinical practice mandatory.[92] All cancer patients who show biomarkers of radiosensitivity should be considered individually in the clinics. In these cases, a different planning approach must be employed toward the intended radio-therapies and their application as a diagnostic or therapeutic method will hugely depend on the extent of the patient's individual radiosensitivity. In all other cancer cases, the chosen clinical approach will be guided and determined by the characteristics of the tumor cells and their potential to undergo or resist apoptosis upon treatment with the weaker ionizing gamma/beta radiation.[28,29,30,32] Alpha-radiotherapies must be considered for all patients with apoptosis-resistant tumors.[27] The life-saving nature of the alpha radiotherapy for patients with difficult-to-treat cancer brings the fastly emerging need for the creation of a sufficient number of production centers. The provision of the necessary radionuclides for future scientific and clinical research will assure the continuous optimization of the alpha therapy.

Authors' Note

E. Nikolova had the idea for the article, collected and processed the relevant literature on the topic and wrote the first draft of the manuscript. D. Tonev, N. Zhelev, and V. Neychev supervised, reviewed, and edited the manuscript.

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