ELSEVIER

Seminars in
RADIATION
ONCOLOGY

Exploiting Gene Expression Kinetics in Conventional Radiotherapy, Hyperfractionation, and Hypofractionation for Targeted Therapy

Adeola Y. Makinde, PhD,[*] Iris Eke, MD, PhD,[*] Molykutty J. Aryankalayil, PhD,[*] Mansoor M. Ahmed, PhD,[†] and C. Norman Coleman, MD[*,†]

The dramatic changes in the technological delivery of radiation therapy, the repertoire of molecular targets for which pathway inhibitors are available, and the cellular and immunologic responses that can alter long-term clinical outcome provide a potentially unique role for using the radiation-inducible changes as therapeutic targets. Various mathematical models of dose and fractionation are extraordinarily useful in guiding treatment regimens. However, although the model may fit the clinical outcome, a deeper understanding of the molecular and cellular effect of the individual dose size and the adaptation to repeated exposure, called multifraction (MF) adaptation, may provide new therapeutic targets for use in combined modality treatments using radiochemotherapy and radioimmunotherapy. We discuss the potential of using different radiation doses and MF adaptation for targeting transcription factors, immune and inflammatory response, and cell "stemness." Given the complex genetic composition of tumors before treatment and their adaptation to drug treatment, innovative combinations using both the pretreatment molecular data and also the MF-adaptive response to radiation may provide an important role for focused radiation therapy as an integral part of precision medicine and immunotherapy.

Semin Radiat Oncol 26:254-260 Published by Elsevier Inc.

Introduction

Over the past 2 decades, the development of and innovations in "omics" technology provide a better understanding of the biological effects of radiation and chemotherapy in normal tissues and tumors, which has in turn led to the improvement in combined modality therapy.[1]

Radiotherapy (RT) delivered in a fractionated regime is based on the differing radiobiological responses of cancer and normal tissues.[2-4] Unlike tumor cells, normal cells repopulate during or shortly after a course of therapy, thus providing an opportunity for the repair of normal tissue damaged due to radiation. The difference in the shape of the radiation survival curve using various radiobiological models from clinical data helps to explain the clinical outcome from both dose size and fractionation scheme.[5,6] Conventional RT (ConvRT) is administered in 1.8-2.2 Gy single fractions per day, 5 days per week for a total of 3-9 weeks, and maximum dose between 60 and 90 Gy.[7-9] In contrast, hyperfractionated RT (HyperRT) is administered in smaller doses of 0.5-1.8 Gy with multiple fractions per day for 2-4 weeks, and hypofractionated RT (HypoRT) as single daily fractions 3-20 Gy with a small number of fractions usually over a week. Overall, advances in technology such as intensity-modulated RT, image-guided RT, stereotactic body RT, stereotactic radiosurgery, and protons and carbon RT have improved the ability to deliver higher radiation dose more accurately to tumors.[3,5] With these technologies, there is still some dose heterogeneity, especially with intensity-modulated RT and image-guided RT, which often by design, include a greater volume of surrounding normal tissue around the target area receiving a smaller overall dose.[1,3,10]

To date, a key determinant for selection of optimal fractionation schedules and dose is the site of tumor being treated. In comparison to ConvRT, HypoRT is the "new

*Radiation Oncology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD.
†Radiation Research Program, National Cancer Institute, National Institutes of Health, Bethesda, MD.
Conflict of interest: None.
Address reprint requests to Adeola Y. Makinde, PhD, Radiation Oncology Branch, Center for Cancer Research, National Cancer Institute (NCI), National Institutes of Health, 10 Center Dr, Building 10—Room B3B406, Bethesda, MD 20892. E-mail: adeola.makinde@nih.gov

http://dx.doi.org/10.1016/j.semradonc.2016.07.001
1053-4296//Published by Elsevier Inc.

kid on the block." There are currently several ongoing and a few complete randomized clinical trials evaluating patient survival, normal tissue effects (acute and late), and several other endpoints of HypoRT or HyperRT or all of these in comparison with ConvRT (Table 1). Recent Phase III reports by Lee et al[8] and Wilkins et al[9] show that HypoRT is not inferior to ConvRT in localized prostate cancer. Consequently, HypoRT could become the standard treatment for localized prostate cancer, as HypoRT may provide the benefit of shorter, potentially less costly, and more efficient treatment schedules and fewer patient visits. HyperRT, on the contrary, has been shown to be superior to ConvRT in head and neck cancer,[11,12] small-cell lung cancer,[13] and non–small cell lung cancer.[14-16]

Although the effects of radiation on key cellular processes and the a21ssociated phenotypic changes (cell cycle arrest, cell death, and DNA repair) are well established in literature, knowledge of the different patterns in gene expression resulting from ConvRT, HyperRT, and HypoRT is limited. Understanding these differences could lead to selective induction of molecular targetable pathways that would enhance tumor killing, potentially using radiation in unique ways by which to improve overall patient outcomes. The use of molecular profiling of tumors treated with fractionated radiation could potentially improve patient outcomes by providing opportunities to select optimal radiation regimens and to allow for identification of targetable pathways and molecules altered because of fractionation. Preclinical evidence shows that fractionated radiation, with doses ranging from 0.5-30 Gy can induce an extensive array of targetable molecules (Table 2). These radiation-induced changes are based on the dose size and number of fractions.[17-30] Selection of optimal fractionated radiation regimens and molecular-targeted therapies resulting from molecular tumor profiling could potentially improve patient outcomes. Other than with immunotherapy,[31,32] there are currently no clinical reports of combined modality therapies exploiting fractionated radiation-induced molecular changes.

Current developments in genomic technology and molecular-targeted therapy allow for classification of patients as part of "precision medicine" and also elucidation of radiation-induced biological changes and selection of effective treatment strategies. The radiation-induced molecular phenotypes are influenced by a possible number of factors such as the stage, site, hypoxic state and the underlying genetic profile of the tumor,[3] type of radiation, and dose and fractionation schedule.[10,33] Ongoing clinical trials may provide data that could potentially identify patient groups that would respond better to specific radiation regimens and combined modality therapies.

In this article, we examine targetable pathways and molecular-targeted therapies in the context of how fractionated radiation (conventional, hyperfractionation, and hypofractionation) regimes can be used to develop unique combined-modality strategies. Our discussion is based on the current literature of fractionated radiation-induced gene expression (Table 2) as follows: (1) transcription factors, NF-κB and STAT1, (2) immune and inflammatory response, and (3) stem cellness and epithelial-to-mesenchymal transition (EMT).

Radiation and Transcription Factors

NF-κB

As a transcription factor that regulates the expression of genes involved in cell differentiation or stemness,[34] cell proliferation, cell death, immune and inflammation responses,[34-36] and the discussion of the role NF-κB plays in cellular response to radiation is essential. Modulation of its expression or activation by radiation has been published in multiple reports.[20,36-39] In 2009, Madhusoodhanan et al[20] showed increased NF-κB activation after fractionated radiation. Poly(ADP-ribose) polymerase[39] and ataxia telangiectasia mutated,[40] proteins with well-established roles in DNA repair, have been reported to function as mediators of radiation-induced NF-κB activation. Although NF-κB has been associated with increased invasiveness, stemness and resistance to therapy, the clear dependence of the "Radiation Survivor"[10] phenotype on its transcriptional activity[20] make it exploitable to combined modalities of radiation and targeted therapies.

Although it may be necessary to use NF-κB inhibitors in long-term therapeutic strategies to enhance tumor cell killing, it should be noted that there is an ongoing discussion regarding the success of molecularly therapies targeting NF-κB, as its prolonged inhibition could be immunosuppressive and compromise normal tissue homeostasis.[41,42] Activity of NF-κB proteins varies depending on the cell type, upstream stimuli, and the cell's microenvironment.[43] As a transcriptional activator, constitutively active NF-κB primarily confers pro-survival, antiapoptotic, or proinflammatory properties or all of these in tumors.[43] Conversely, NF-κB activation resulting from treatment with some DNA damaging agents have been reported to be transcriptionally repressive.[44] As reviewed by Chaturvedi et al[43] on NF-κB activation in cancers, "one size does not fit all." A well-defined mechanism for its constitutively or radiation-induced activation is necessary for the development of effective combined modality strategies that use radiation and molecular-targeted NF-κB therapy. Alternatively, specific radiation-induced NF-κB, coregulators, and other downstream effector pathways and molecules could also be targeted, as they are more likely to spare normal tissue and be specific to the tumors.[45,46]

STAT1

STAT1 is a transcription factor that promotes the expression of genes involved in cellular differentiation, cell proliferation, and cell death. It is also an essential signaling molecule in response to interferon (IFN) stimulation. Tsai et al[18] report the upregulation of STAT1 expression and other IFN-related genes after fractionated radiation. Our group has also reported a fractionated radiation-induced upregulation of STAT1 and IFN-inducible genes and proteins in primary human normal endothelial[23] and a prostate cancer model.[22,25] Like NF-kB, STAT1 and IFNg-related genes have been implicated in chronic inflammation,[18] increased resistance to therapy, and an aggressive invasion or metastatic phenotype.[47-79]