Biological consequences of radiation therapy: a new era of normal tissue, cancer and immune biology

Robert J. Griffin, PhD
University of Arkansas for Medical Sciences
Outline:

Disclosures: Nothing to disclose

FOCUS: Two recent NIH/NCI workshops on Radiation Biology

1. Recognizing the need to continued Radiation biology insight

2. Sensing a shift in the field/discipline to recognize radiation as a tool/drug in some contexts more than ever before
REVIEW

The Future of Radiobiology

David G. Kirsch, Max Diehn, Aparna H. Kesarwala, Amit Maity, Meredith A. Morgan, Julie K. Schwarz, Robert Bristow, Sandra Demaria, Iris Eke, Robert J. Griffin, Daphne Haas-Kogan, Geoff S. Higgins, Alec C. Kimmelman, Randall J. Kimple, Isabelle M. Lombaert, Li Ma, Brian Marples, Frank Pajonk, Catherine C. Park, Dörthe Schaeue, Eric J. Bernhard

Abstract

Innovation and progress in radiation oncology depend on discovery and insights realized through research in radiation biology. Radiobiology research has led to fundamental scientific insights, from the discovery of stem/progenitor cells to the definition of signal transduction pathways activated by ionizing radiation that are now recognized as integral to the DNA damage response (DDR). Radiobiological discoveries are guiding clinical trials that test radiation therapy combined with inhibitors of the DDR kinases DNA-dependent protein kinase (DNA-PK), ataxia telangiectasia mutated (ATM), ataxia telangiectasia related (ATR), and immune or cell cycle checkpoint inhibitors. To maintain scientific and clinical relevance, the field of radiation biology must overcome challenges in research workforce, training, and funding. The National Cancer Institute convened a workshop to discuss the role of radiobiology research and radiation biologists in the future scientific enterprise. Here, we review the discussions of current radiation oncology research approaches and areas of scientific focus considered important for rapid progress in radiation sciences and the continued contribution of radiobiology to radiation oncology and the broader biomedical research community.
Radiation Research Program
Division of Cancer Treatment and Diagnosis

Defining the Shades of Gy:
Utilizing the biological consequences of radiation therapy in the development of new treatment approaches

SEPTEMBER 11-12, 2017
NCI SHADY GROVE CAMPUS, ROCKVILLE, MD
Evolution of High Dose Ablative Radiation

LDR → HDR → SBRT

EBRT
Concept: Target induction with radiotherapy

Radiation-induced target expression and activation

Targeted drugs

Cell death

Analysis of the radiation-induced molecules
(Gene microarray, phosphoproteome array...)

Eke, I et al. NCI Radiotherapy branch
Selected topics:

• **Response to conventional fractionation**
  • senescence? Resistance?
  • Induction of targets or proteins to allow additional therapy to act (targeted drugs, immunotherapy, others?)

• **Response to SRS/SBRT-like dose regimens**
  • Normal tissues: Pelvic, head and neck
  • Linear quadratic at high doses makes sense, but why?

• **Vascular/physiological changes underlying response and relationship to immunotherapy outcomes**
  • Indirect cell death
  • Abscopal effects

• **New strategies/modalities, old ideas?**
  • Spatial fractionation/flash
  • Tumor treating fields
Figure 22.7. Isoeffect curves in which the total dose necessary for a certain effect in various tissues is plotted as a function of dose per fraction. Late effects are plotted with solid lines, acute effects with dashed lines. The data were selected to exclude an influence on the total dose of regeneration during the multifraction experiments. The main point of the data is that the isodoses for late effects increase more rapidly with a decrease in dose per fraction than is the case for acute effects. (From Withers HR: Cancer 55:2086, 1985, with permission.)
Therapy-induced apoptosis and tissue damage connected to tumor recurrence

Huang and Li et al, Nature Medicine 2011

Figure 1 Huang et al.\textsuperscript{5} show that cytotoxic oncology therapies induce caspase 3 activation, which, in turn, can generate competing effects on apoptotic cell death and stimulation of tumor growth. Thus, the balance between these two competing processes determines the final outcome for the tumor: shrinkage or recurrence. AA, arachidonic acid; iPLA\textsubscript{2}, calcium independent phospholipase A\textsubscript{2}.
Normal tissue damage also influenced by dual roles of caspases: stem cell proliferation/regeneration

C-Y Li et al, Science Signaling, 2010
Figure 4. Radiation as an Immune Modulator.

A simplified depiction of the immune effects of localized irradiation is shown. Radiation can affect the immune state of a tumor in many ways. Irradiation of tumor vasculature can result in expression of adhesion molecules, such as intercellular adhesion molecule 1 (ICAM-1). Irradiated tumor cells increase the expression of major histocompatibility complex class I (MHC-I) and Fas. Tumor antigens are released from dying tumor cells, and injured and dying cells may translocate calreticulin to the cell surface. Factors that can activate dendritic cells, such as damage-associated molecular patterns (DAMPs), high-mobility group box 1 protein (HMGB1), and ATP, are also released from irradiated tumor cells. Dendritic cells present tumor-cell antigens to naive T cells, facilitating their conversion to cytotoxic effector cells, which may recognize tumor cells, a process that may also be enhanced by increased MHC-I expression. These events may be highly dependent on the radiation dose, target, and volume and may vary as a function of time after radiation exposure.
Normal tissue next to tumor exists in a state between healthy and cancerous. Inflammatory phenotype long after therapy.

Nature Communications 2017: Normal tissue adjacent and tumor transcriptome
Aran and Butte et al.

ASTRO Annual Refresher Course • Fort Lauderdale Marriott Harbor Beach Resort & Spa • March 2-4, 2018 • #REFRESHER18
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  • Spatial fractionation/flash
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Stromal cells important in response of normal tissue and tumor tissue to radiation.

1. Vascular response dictates many of the systemic effects of SBRT doses (inflammation, immune system access, indirect cell death)

2. Crosstalk between damaged cells and tissue/tumor regeneration an important factor in results

Stereotactic Body Radiation Therapy
1-5 fractions of 6-24 Gy each; daily, every other, weekly

Many normal tissues have lower total dose tolerance
Figure 18.8. Left: Histology of normal testis. Right: Histology of testis 35 days after a dose of 9 Gy (900 rad) of γ-radiation. Some tubules are completely devoid of spermatogenic epithelium and some are not. (Sertoli’s cells persist in the tubules sterilized of spermatogenic cells.) Foci of spermatogenesis can be derived from single surviving stem cells. (Magnification ×200.) (From Withers HR, Hunter N, Barkley HT Jr, Reid BO: Radiation survival and regeneration characteristics of spermatogenic stem cells of mouse testis. Radiat Res 57:88–103, 1974, with permission.)
Prostate Tissue after SBRT

37.5 Gy / 5 fx then urinary retention 1 year later requiring TURP

10x - glandular atrophy with no evidence of residual cancer, fibrosis, or necrosis

40x – general hyperplasia and chronic inflammation, nuclear and cytoplasmic cellular changes

Chronic bladder changes include loss of submucosal vessels and fibrosis: 20 Gy, endothelial dysfunction.

Hypoxia ensues 1-2 days after single high dose radiation (20 Gy)

Song, CW, Griffin RJ et al, Red Journal, 2015
Indirect cell death from vascular damage in SBRT

Song CW, Griffin et al, IJROBP: 2015
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Tumor is filled with normal tissue (stroma):

Photoacoustic mapping of ear vasculature and photofluorescent scanning of tumor mass co-opting the normal tissue
Summary:
irradiated endothelium conduit between tissue and inflammatory system

Choi and Pober et al. Annu. Review Immunology 2004
Immune cell activation by radiation

Radiation Research, 2014

FIG. 1. Schematic representation of the envisioned mechanism of radiation-primed immunotherapy of advanced cancer.
Barriers to Radiation-induced *In Situ* Tumor vaccination

*Frontiers in Immunology, 2017*

Erik Wennerberg1, Claire Lhuillier1, Claire Vanpouille-Box1, Karsten A. Pilones, Elena García-Martínez, Nils-Petter Rudqvist, Silvia C. Formenti and Sandra Demaria

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**Diagram Description**

**A**
- Irradiation of tumor
- Increased VEGFA level in hypoxic tumor
- Treg proliferation
- T cell proliferation
- Hypoxia fine-tune TAM M2 phenotype
- CTLA-4, PD-1, TIM-3, LAG-3
- TAA-specific killing
- Release of TAA
- Release of DAMPs
- TUMOR-DRAINING LYMPH Node
- Tumor cell
- CD8+ T cell
- Treg
- Immature DC
- TGFβ
- Activation of TGFβ
- TGFB inhibits DC maturation
- Mature DC
- Cross-presentation of TAA

**B**
- Increased VEGFA level in hypoxic tumor
- Treg proliferation
- T cell proliferation
- CD8+ T cell
- DAMPS
- TUMOR-DRAINING LYMPH Node
- Tumor cell
- CD8+ T cell
- Treg
- CD4+ T cell
- CD48 T cell
- TGFB
- TGFβ converts naive CD4+ T cells into Treg
- CD4+ T cell
- TAM polarization [M1 → M2]
- Activation of TGFβ
- TGFβ inhibits DC maturation
- Mature DC
- Cross-presentation of TAA

**C**
- CCL2 recruits monocytes via CCR2
- TAM polarization [M1 → M2]
- CSF1 binds CSFR1 on TAMs
- Increased CCL2 level
- Increased CSF1 level
- Tumor cell
- TAM differentiation
- TAM polarization [M1 → M2]

**D**
- TGFβ converts naive CD8+ T cells into Treg
- CD8+ T cell
- TAM polarization [M1 → M2]
- TGFβ
- Tumor cell
- TGFβ converts TAN N1 to N2
- Activation of TGFβ

**E**
- Adenosine suppresses DCs and CD8+ T cells
- Adenosine
- ATP
- Adenosine
- ATP
- Tumor cell
- TGFβ
- Treg proliferation
- TAM polarization [M1 → M2]
- CD8+ T cell
- CD8+ T cell
- CD8+ T cell
- ATP
- DC recruitment and activation

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**Notes**

- Barriers to *In Situ* Tumor vaccination
- Radiation-induced immune response
- Role of TGFβ and VEGFA
- Tumor microenvironment and immune cell interactions
- Immune cell polarization
- Tumor-specific antibodies

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**References**

• Local control depends on immune contribution

• Optimal dose per fraction when combined with immunotherapy

• Defining “abscopal” effects
T cells contribute to the response to radiotherapy

<table>
<thead>
<tr>
<th>FSA Tumor</th>
<th>TCD$_{50}$ values</th>
<th>Metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal mice</td>
<td>30.0Gy (28.5-32.4)</td>
<td>1%</td>
</tr>
<tr>
<td>Immunosuppressed (6Gy)</td>
<td>50.8Gy (47.6-54.3)</td>
<td>4%</td>
</tr>
<tr>
<td>T cell deprived mice</td>
<td>64.5Gy (62.0-67.1)</td>
<td>79%</td>
</tr>
</tbody>
</table>

Stone et al., JNCI 63:1229, 1979
In vitro assay for RT-induced ICD
(Golden et al. OncoImmunology 2014)

OncoImmunology 3, e28133; January 2014; © 2014 Landes Bioscience

Is tumor (R)ejection by the immune system the “5th R” of radiobiology?

Encoue B Golden and Silvia C Formenti*

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ARTICLE

Received 27 Mar 2017 | Accepted 12 Apr 2017 | Published 9 Jun 2017

DNA exonuclease Trex1 regulates radiotherapy-induced tumour immunogenicity

Claire Vanpouille-Box¹, Amandine Alard²,†, Molykutty J. Aryankalayil³, Yasmeen Sarfraz¹, Julie M. Diamond¹, Robert J. Schneider², Giorgio Inghirami⁴, C. Norman Coleman³, Silvia C. Formenti¹ & Sandra Demaria¹,⁴

DOI: 10.1038/ncomms15618

OPEN
The window of radiation immunogenicity is determined by dsDNA vs Treg1
RT failure to “cure” = pre-existing distant micrometastasis that systemic therapy can not control

Radiotherapy failures to “cure” can also be ascribed to inadequate abscopal effects on micrometastasis
Different pneumonitis patterns related to anti-PD1
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‘Lattice/GRID’ irradiation promotes local and abscopal effects to greater extent than open field: increased tumor specific cytokines

Kanagavelu and Ahmed et al.
Radiation Res 2014
Typical MLC segments or block used for GRID
FLASH: Ultrahigh dose rate, spatial fractionation with microbeams

Maximizing target dose while maintaining normal tissue integrity: interspersing MRT fields at ESRF Grenoble, FR
Microbeam spatial fractionation suggests benefits of improved normal tissue response.

Fig. 1. Horizontal section of the cerebellum of a piglet of 15 months after irradiation with a skin entrance dose of 300Gy. Some cells and their nuclei directly in the path of microbeams were destroyed. There was no tissue destruction present, nor were there signs of hemorrhage. The paths of the microbeams appear in the section as thin, white horizontal parallel stripes, which are more easily visible in the insert. Beam width ~27μm, spacing ~210μm.
16 d after MRT; cerebellum

16 d after MRT; cerebellum, evidence of vascular bridging/repair
Maintenance Therapy With Tumor-Treating Fields Plus Temozolomide vs Temozolomide Alone for Glioblastoma: A Randomized Clinical Trial

CONCLUSIONS AND RELEVANCE In this interim analysis of 315 patients with glioblastoma who had completed standard chemoradiation therapy, adding TTFFields to maintenance temozolomide chemotherapy significantly prolonged progression-free and overall survival.

Figure 2. Survival Curves for Patients Included in the Intent-to-Treat Population

<table>
<thead>
<tr>
<th>No. at risk</th>
<th>Progression-Free Survival From Randomization, mo</th>
<th>Overall Survival From Randomization, mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTFFields plus temozolomide</td>
<td>210</td>
<td>210</td>
</tr>
<tr>
<td>Temozolomide alone</td>
<td>105</td>
<td>105</td>
</tr>
</tbody>
</table>

Survival analyses on time from date of randomization until tumor progression, death, or last follow-up (censored patients) according to the Kaplan-Meier method. The small vertical ticks on the curves indicate censored patients. HR indicates hazard ratio; TTFFields, tumor-treating fields.
Key Points/Summary

• Conventional fractionation and hypofractionation/SBRT have distinct and significant biological effects on tumor and normal tissue that influence efficacy of other treatments, recurrence or metastasis.

• Proper sequencing of therapy due to specific biological reactions of the tumor and normal tissue may be important to increasing impact.

• Improving the immune system’s ability to process and act upon tumor antigens can be accomplished with high dose regimens and abscopal effects are observed in many cases. Optimizing radiation delivery is key.

• Other approaches, whether with high dose rate, spatial fractionation or other energy modalities (treating fields, thermal dosing) may have valuable roles to play in enhancing tumor control.