Box S5 | Spotlight on the ionizing radiation proteome

Radiotherapy is an essential standard-of-care component for a large number of cancer patients, either as neoadjuvant, definitive, adjuvant or palliative therapy, and is often combined with chemotherapy, targeted or biologic therapies. A joint task force of the UK-based National Cancer Research Institute, NCRI and its Clinical and Translational Radiotherapy Research Working Group, CTRad119, estimated that 60% of cancer patients receive radiotherapy as part of their treatment and that 40% of cancer cures include radiotherapy either as monotherapy or in combination with other treatments1. According to the WHO, “cancer is the second leading cause of death globally, and was responsible for 8.8 million deaths in 2015”. Given its global impact and the large number of patients who are likely to receive radiotherapy, genomic and proteomic changes in the context of radiotherapy are likely to represent unexplored opportunities that need to be considered when paired with drug therapies.

Unfortunately, our appreciation of the radiation genome and proteome following irradiation is significantly understudied, and perhaps even less understood than Tdark proteins. One in vitro study examined 10,174 genes in immortalized B-cells following irradiation and suggested that up to one-third of the genome is radioresponsive2,3. Of the 447 genes with significant fold changes, 26 are Tclin, 61 Tchem, 268 Tbio, and 92 are Tdark, respectively (data3 from GSE26835, available in Supplementary information S3 (table)). Though limited, this set is enriched in Tbio/Tdark proteins, suggesting that the ionizing radiation proteome may be understudied.

Compared to understanding the effect of drugs on specific proteins, radiotherapy may be a more complex problem: For example, up- or down-regulation of ionizing-radiation sensitive genes, or altered post-translational modification of proteins following radiotherapy can significantly affect drug-protein interactions, which in turn influence drug treatment outcomes. In part, this may explain why chemotherapy is often paired with radiotherapy. Following radiotherapy, some of the protein interactions and the changes in gene expression have been characterized, but the vast majority of these changes appear to be understudied. Layering gene and protein changes following oncologic radiotherapy on top of the existing TDL categories are likely to require a different type illumination, for a category that we label neo-Tdark.rad. Conceivably, the ionizing radiation proteome represents a smaller but potentially fruitful category in need of further study. Below we provide a contemporary example of how a better appreciation of neo-Tdark.rad may enhance existing oncologic therapy.

The use of immunotherapy in melanoma has demonstrated the potential to enhance patient survival in a significant manner, which has led researchers and clinicians to explore immunotherapy alone or in combination with other treatment regimens in numerous other tumor types. The observation of the abscopal effect in melanoma, where ipilimumab, an anti-CTLA-4 monoclonal antibody was combined with stereotactic body radiotherapy, has demonstrated that local ablative radiotherapy could lead to a systemic tumor response4. Preclinical data suggests that radiotherapy either with or without immunotherapy leads to changes in the immune microenvironment, with increased NK-T cells, CD8 effector cells and antigen-presenting cells5,6. At the gene and protein levels, the microenvironment has been shown to have alterations in chemokines allowing for effector T-cell extravasation7–9, a conversion of the M2 (tumor associated macrophages) to a M1-anti-tumor, pro-inflammatory macrophage phenotype10, up-regulation of pro-inflammatory cytokines such as IL-1β and TNF-α7,11–13, in addition to tumor specific up-regulation of major histocompatibility complex (MHC) Class I co-stimulatory immune
markers and ligand-mediated death receptors\textsuperscript{6,14-18}. Furthermore, presentation of tumor neoantigens following radiotherapy may represent another critical piece where these novel antigens presented on MHC Class I receptors may allow for enhanced immune recognition and destruction of the tumor\textsuperscript{19-21}. Tumor neoantigens represent key protein mutational spectra that could be used to stimulate the immune response and are distinct from normal host proteins. Neoantigen presentation as a function of radiotherapy-induced gene/protein changes in the tumor and local microenvironment are likely needed to stimulate the necessary anti-tumor responses seen in preclinical models. However, the genes described above are likely to represent only a fraction of the events that occur between the tumor and local immune microenvironment, and do not take into account the potential radiation-mediated changes on blood vessels, tumor-associated fibroblasts and myofibroblasts, pluripotent stem cells or cancer stem cells, which similarly reside within this complex microenvironment. Shedding light on \textit{neo-T_{dark,rad}} is likely to provide additional insights for clinicians and scientists, which could lead to more rational combinations of radiotherapy and immunotherapy to potentially enhance treatment and survival outcomes in patients.

**Resources**


**References**

8. Matsumura, S. \textit{et al.} Radiation-induced CXCL16 release by breast cancer cells attracts effector T


