Molecular biology: the key to personalized treatment in radiation oncology?

Pratikhya Patel[a]

[a]Department of Biomedical Engineering, National Institute of Technology Raipur, India

Abstract

We now know a lot more about what causes cells and tissues to be resistant or vulnerable to radiation than we did 20 years ago. Novel approaches in molecular biology have made significant contributions to our knowledge of signaling circuits. Before the period of "New Biology," radio responsiveness was described in terms of physiological parameters known as the five Rs. repair, repopulation, reassortment, reoxygenation, and radiosensitivity. Only the function of hypoxia proved to be a reliable predictive and prognostic sign, although radiation regimens were altered in terms of dosage per fraction, fraction size, and total duration, in ways that are being used in clinical practice today. Around two decades ago, the first molecular methods were used in radiobiology, quickly revealing the presence of genes/proteins that respond to and impact the cellular result of irradiation. The later development of microarray-based screening tools has revealed that a huge number of genes fall into this group.Using gene expression and proteomic techniques, we can now create a sufficiently strong molecular signature predicting a radioresponsive phenotype. Parallel to these advances, functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) can now identify particular biological components like hemoglobin and glucose, showing a 3D map of tumor blood flow and metabolism. The ability to extend this skill to the proteins of the molecular signature that govern radiosensitivity will be critical to tailored radiotherapy.

Keywords: Molecular biology; oncology; radio-biology

1 INTRODUCTION

Molecular biology is the study of the molecular foundation of biological activity inside and between cells, including molecular production, modification, processes, and connections. Molecular biology is the study of the chemical and physical structure of biological macromolecules.

Over the last 20 years, advances in molecular biology have presented us with a wonderful array of tools that have improved our knowledge of how tumors and normal tissues respond to radiation damage. As these approaches become more advanced, their implementation should, in principle, provide the potential to increase radiation effectiveness.

However, when we look at how radiotherapy is done now, we see a discipline built on 100 years of practice-based, empirical development, which has lately been bolstered by amazing breakthroughs in dose administration and image-guided treatments. These advancements have led to a stage where dosage deposition is already extremely customized, with a tolerance of 2

2 BASIC RADIOBIOLOGY AND THE FIVE Rs

The interaction of high-energy X-ray photons with tissue causes the ejection of fast electrons from molecules (mostly water), which generates a broad spectrum of secondary electrons, photons, and free radicals. The most reactive of these radicals, OH, can generate more radicals of macromolecules. If they are required for cellular function, as in the case of DNA, cell biology may be disrupted, perhaps leading to cell death.

These processes have been recognized for at least 50 years, but there is still a lot to learn about the entire spectrum of harmful species caused by an incoming high-energy photon. It is also well recognized that the early effects of free radical generation at the cellular level may be significantly altered by two key conditions: oxygen tension and the concentration of free radical scavengers such as glutathione. More sophisticated mechanisms including DNA repair and activation of death signaling pathways determine how the cell handles and responds to its accumulated damage.Tissue-level consequences are far more complicated, and their significance to radiation oncology was first defined in terms of the four Rs of Radiobiology: DNA repair (enzymic), reoxygenation (of previously hypoxic cells), repopulation (cell proliferation), and redistribution (to phases of the cell cycle with

differing radiosensitivity). Later, it was recognized that sensitivity between cell lines might vary for causes other than the four Rs, therefore a fifth R, inherent radiosensitivity, was introduced.These five principles might explain the heterogeneity in cellular and even tissue response in general terms, but they usually give us very little about the processes governing the response to radiation exposure at the molecular level. The more recent use of molecular approaches to this area has introduced further complication, which has been well explored.[2]

3 PREDICTIVE TESTING BASED ON TRADITIONAL RADIOBIOLOGY

Each of the five Rs has the potential to contribute a significant dose modifying factor (2–5, but the majority are not technically dose modifying) to the final outcome of radiation. As a result, it stands to reason that assessing these factors in individual patients might have huge predictive value.

However, attempts to construct predictive assays based on the assessment of these five factors have met with varying degrees of success. However, it is important to note that the science underpinning the five Rs was designed to provide a framework to facilitate understanding of new occurrences in radiation biology rather than to forecast outcomes. Furthermore, the lack of effectiveness might be attributed to the fact that there are only little quantitative differences between many normal tissues and human tumors, as well as a high degree of overlap in their heterogeneity.[3]

4 Hypoxia

For many years, it has been known that hypoxia, as evaluated by several direct and indirect approaches, closely correlates with the success of all cancer therapy. This is not confined to individuals receiving cytotoxic treatments that are known to be oxygen-dependent, such as radiation. There is now compelling evidence that hypoxia influences cancer prognosis through a variety of pathways, including increased inflammation, promotion of malignant development, and a direct reduction in the efficacy of therapy. The use of molecular tools has substantially improved our understanding of how these events are mediated, especially the function of the master regulator HIF-1.

Increased staining for HIF-1 alpha expression and the major downstream mediator, vascular endothelial growth factor (VEGF), were significant predictors of a shorter time to biochemical failure in prostate cancer patients treated exclusively with radiation or surgery. Furthermore, a 99-gene hypoxia gene signature obtained from head and neck tumors, as well as the meta-signature identified by the same group, maybe radiobiologically significant in terms of stratifying patients for radiation treatment.[4]

5 Cell Kinetics

Cell cycle characteristics vary greatly across individual human tumors, therefore it stands to reason that the duration of the cell cycle or its discrete phases should affect radiation outcome. After all, we know that cells are more vulnerable to radiation at different stages of development, and that proliferation is related to the rate of tumor recurrence during and after therapy. Few studies have been big enough to provide statistically meaningful results. One multivariate investigation of head and neck cancer patients from 11 different European centers, on the other hand, demonstrated unequivocally that no cell kinetic measure could be depended on to predict local control.[5]

6 Intrinsic radiosensitivity

It may appear obvious that the intrinsic radiosensitivity of tumors or even normal cells generated from cancer patients should correspond with treatment success. This has not only proven difficult to show, but different endpoints for DNA damage do not correlate well with one another.

The most thorough investigations have involved measuring the surviving proportion of tumor cells from cervical cancer patients in vitro after 2 Gy of radiation (SF2). A clear link was discovered, but it took the careful determination of the proper cut-off value for SF2 (0.42) to distinguish between excellent and poor results. In head and neck cancer, a similar outcome was observed, but not consistently.

However, the limits of these techniques are likely to be both technological and biological. Primary cultures from human tumors are extremely difficult to develop, and even when they do form colonies, plating efficiency is just about 1The widespread recognition of the role of the double-strand break as a major DNA lesion dictating cell fate following irradiation has led to an examination of its repair as a surrogate measure of radiation sensitivity (see Hennequin et al 2009 for a review). In one investigation of 10 human tumor cell lines, DNA end-binding complexes (indicative of repair beginning) were associated with SF2 in primary fibroblast cultures and human

tumor cell lines. The scoring of chromosomal abnormalities may be a more conclusive technique, and there is convincing evidence from studies on human cell lines that they may predict cell survival.[6]

7 FUNCTIONAL GENOMICS AND MOLECULAR RESPONSES TO RADIATION EXPOSURE

We now know that, like other DNA-damaging agents, ionizing radiation causes a complicated process of up and down-regulation of genes that interact through several pathways. Fornace and colleagues pioneered this field more than 20 years ago when they used cDNA library screening to reveal that important genes, GADD45A and p21(CIP1/WAF1), were up-regulated by ionizing radiation.Around this time, the key role of the p53 regulator became clear, and studies revealed that many pathways downstream of this master regulator must be crucial in the response of cells to ionizing radiation. GADD45, CDKN1A, and MDM2 are all-important proteins. Initial research revealed that they were caused by a high dose of X-rays (20 Gy), but not in all cell lines and not in a predictable, p53-dependent manner.

With the development of microarray technology, new tools for identifying changes in gene expression in response to ionizing radiation were available. The initial application of this technology to radiation response utilized significant (20 Gy) doses and permitted the mapping of interactions between several genes, many of which were unknown and many of which were controlled by p53. The same group demonstrated that microarray analysis may identify differential gene expression in blood cells in response to radiation doses of 2 Gy or less in the clinically relevant therapeutic range. Many of these genes, which are known to be involved in apoptosis or cell cycle checkpoints, are dose rate dependent.

Microarray analysis has also proven useful in elucidating the mechanisms involved in bystander death in unirradiated cell populations that are close to and share a shared medium with irradiated cell populations. This includes evidence that connexin 43, a protein involved in gap junction communication, and cyclooxygenase are involved. Bystander reactions can also be communicated through the media that has allowed irradiated cells to develop. p53, p21, MDM2, CDC2, Cyclin B1, and RAD51 are all strongly altered in bystander cells, despite the lack of complete profiling.

A significant amount of time and effort has gone into studying the expression of specific genes and gene products as indicators of biological response to radiation. This prompted hopes that biomarkers might be able to predict outcomes more precisely than previous techniques that focused on size, distribution, stage, and grade. While biomarkers for chemotherapy response, such as HER2/neu in breast cancer, are becoming more well recognized, their use in radiation planning is far less developed. The function of the cell cycle and DNA repair regulator EGFR in determining radiosensitivity in tumors from various locations (colorectal, brain, and head and neck) has been demonstrated, however, the association is not entirely constant.Members of the p53 gene family and genes controlled by p53 have shown similar relationships. Several genes, including cyclin D1, TS, TP, DPD, and Her-2/neu, have also been demonstrated to be predictors of response, survival, and recurrence in patients treated with radiochemotherapy for squamous cell carcinoma of the esophagus using quantitative RTPCR methods.

As the number of genes associated with radiation response grows, numerous groups have employed microarray analysis to generate a global signature suggestive of radio responsiveness/resistance in colorectal cancer. Several additional gene signatures have been identified about the radioresponse of cervical, breast, and head and neck tumors, as well as, more recently, breast cancer. However, the overlap between these gene signatures is modest, the statistical significance has been called into question, and no large-scale, randomized studies have yet been published to thoroughly confirm the use of any of these signatures in the various tumor types. A gene expression model that predicts intrinsic radiosensitivity and treatment response in a wide range of cancer patients is required. A good strategy might be to look for differences in the expression of key genes known to play important roles in processes that affect biological responses to radiation. In 48 human rectal, head and neck, and esophageal cancer cell lines, radiosensitivity was modeled as a function of gene expression, tissue of origin, ras (mut/wt), and p53 status (mut/wt). This group found ten important or "hub" genes implicated in pathways critical to cell signaling control.

We are used to seeing well-defined correlations (e.g., linear quadratic) between radiation dosage and cell damage endpoints like cell survival, but it became obvious early on that changes in the expression of radiation-modulated genes do not often reflect these relatively straightforward dependencies. Several studies, for example, have found a transcriptional response that is only seen at low dosages.

These gene expression studies have aided in the identification of pathways of interest, but we must remember that cellular responses are mediated at the protein level, so translational regulation, post-translational modification, and protein degradation must add additional levels of complexity to the genomic responses identified by microarrays.

In addition, gene expression is being used as a radiobiological endpoint. Other researchers have employed genotyping to correlate germ-line single nucleotide polymorphisms (SNPs) in normal and tumor tissue in order to measure normal tissue radiation sensitivity and tumor response. The research, which focused on tumor response, revealed that genetic variations related to DNA repair and apoptosis appear to be essential. Four big studies are underway to thoroughly validate markers for normal tissue radiation toxicity, but large-scale validation of SNPs

that might be effective predictive markers of tumor radio-responsiveness is still absent.However, studies such as the normal tissue radiation toxicity (RAPPER) research may allow radiation-tolerant patients to receive a higher tumor dosage, boosting their chances of local recurrence-free survival. Furthermore, if a link exists between tumor and normal tissue radiosensitivity, genetic profiling will be much more useful in the management of radiotherapy patients.[7]

8 APPLICATION TO ARCHIVED SAMPLES

Many hospitals have large collections of formalin-fixed tumor and normal tissue samples with known patient outcomes. This resource should theoretically be susceptible to genomic, RNA, and proteomic investigation, allowing for the discovery of critical pathways involved in the radiation response of tumor and normal tissue to therapeutically relevant irradiation regimens. To present, the number of research that has used this material is very limited, owing to the difficulty of analyzing samples containing extremely minute quantities of badly degraded DNA.

However, techniques for profiling SNPs in DNA from paraffin-embedded tissue have been established. The issue of sample size has been addressed by the use of whole gene amplification methods, which may be utilized to detect changes in gene copy numbers. Bead arrays have also been used to determine DNA methylation and gene silencing in a unique, high throughput approach.

Changes in gene expression at the RNA level can also be determined in paraffin-embedded sections using real-time PCR in conjunction with proteinase digestion, which can then be enhanced by laser-assisted microdissection to focus on regions of interest and maximize the amount of message in the sample.

Protein analysis may also be used to extract information from archival material. Immunohistochemical staining of tissue sections with specific antibodies can be successful when combined with antibody retrieval methods such as heating. This method may also be used to remove proteins from fixed material in preparation for 2D gel electrophoresis.

Alternatively, paraffin removal and enzyme digestion have increased the efficiency of matrix-assisted laser desorption/ionization liquid chromatography/mass spectrometry analysis. Protein interactions play a critical role in the activation of pathways in response to cytotoxic stimuli like radiation. These interactions may be detected using a technique that takes advantage of the proximity ligation of oligonucleotides coupled to certain antibodies.

This type of data, obtained through gene expression and proteomic approaches, has made it possible to link the regulation of specific pathways to radiotherapy outcomes. This opens up the possibility of using that knowledge to inform patient care in the form of personalized molecular therapy. A new study discusses the role that proteomics, especially mass spectrometry, might play in translating cell biology into therapeutic practice.[8]

9 BIOMARKERS AND FUNCTIONAL IMAGING

In many ways, using non-invasive functional imaging technologies to accomplish "biologically conformal therapy" is the holy grail of customized treatment planning. This will necessitate the use of trustworthy biomarkers in conjunction with functional imaging techniques such as PET or MRI. While we are still a long way from being able to detect gene or product expression levels in vivo using remote imaging methods, diffusion-weighted MRI (DCE-MRI) has been used successfully as a predictor of the response of the brain and colorectal tumors, while [18F]FDG-PET data was of prognostic value in the lung, gastric, esophageal, liver, breast, head and neck, and cervical cancer.

However, the evidence that biomarkers or functional imaging are preferable to anatomical imaging utilizing CT inpatient treatment is still lacking, at least in the case of head and neck cancer.

Molecular approaches are allowing us to gain a better understanding of the signaling networks that govern normal and malignant cell radiation responses. Simultaneously, developments in imaging technology, notably MRI and PET, are expanding the number of functional indicators that can be assessed in tissues in real-time.

However, there is still a long way to go before noninvasive tumor evaluation based on genetic markers for particular pathways is conceivable. The mathematical complexity of combining a large number of parameters in each cell with different levels of expression according to each unique position within a tumor, as well as the problem of temporal changes during protracted treatments, would have been beyond the computational power available only a few years ago. Massive processing power can now be applied to the problem, and when paired with improved computational approaches, there is some hope of success.

However, complicated algorithms are only as good as the inputs they get, and giving weights to the various factors that would be required, even if they can be measured reliably, remains a challenge. Epigenetic changes caused by concurrent vascular or immunological diseases, for example, may outweigh the expectations of solely genetic characterizations.

A completely integrated approach to customized therapy will take many years to implement in clinical practice. In the meanwhile, components of this notion may be useful in treatment planning soon.[9]

10 BACKGROUND AND PURPOSE

We are making remarkable progress in understanding the fundamental processes behind cancer onset and progression. The application of this new information to daily clinical radiation oncology practice may not be obvious at first. To properly comprehend the therapeutic significance of the new biology, it is required to be familiar with a few ideas from molecular biology and biochemistry.[10]

11 CONCLUSION

The breakthroughs in molecular biology have a direct influence on radiation oncologists' roles in the clinic. While key novel medicines are still in the lab, they will almost certainly play a big role in patient care and cancer prevention in the not-too-distant future. Given the central role of radiation oncologists in cancer management, a basic understanding of molecular biology techniques and their application is required so that we can keep up with our colleagues and patients and, as a specialty, actively participate in improving the outcomes of cancer patients.

12 References

[1]Masoodi, K. Z., Lone, S. M., Rasool, R. S. (2021). Introduction to molecular biology techniques. In Advanced Methods in Molecular Biology and Biotechnology.

[2]Suwiński, R., Gawkowska-Suwińska, M. (2001). Radiobiologic basis for using 131I to treat patients with thyroid cancer. In Wiadomości lekarskie (Warsaw, Poland: 1960): Vol. 54 Suppl 1.

[3]Romeiko, X. X., Guo, Z., Pang, Y., Lee, E. K., Zhang, X. (2020). Comparing machine learning approaches for predicting spatially explicit life cycle global warming and eutrophication impacts from corn production. Sustainability (Switzerland), 12(4).

[4]Telarovic, I., Wenger, R. H., Pruschy, M. (2021). Interfering with Tumor Hypoxia for Radiotherapy Optimization. In Journal of Experimental and Clinical Cancer Research (Vol. 40, Issue 1).

[5]Tubiana, M. (1982). L.H. Gray medal lecture: Cell kinetics and radiation oncology. International Journal of Radiation Oncology, Biology, Physics, 8(9).

[6]Azimian, H., Dayyani, M., Bahreyni Toossi, M. T., Mahmoudi, M. (2018). Bax/Bcl-2 expression ratio in prediction of response to breast cancer radiotherapy. Iranian Journal of Basic Medical Sciences, 21(3).

[7]Disler, R. T., Gallagher, R. D., Davidson, P. M., Sun, S.-W., Chen, L.-C., Zhou, M., Wu, J.-H., Meng, Z.-J., Han, H.-L., Miao, S.-Y., Zhu, C.-C., Xiong, X.-Z., Reis, M. S., Sampaio, L. M. M., Lacerda, D., De Oliveira, L. V. F., Pereira, G. B. M., Pantoni, C. B. F., Di Thommazo, L., ... Mistraletti, G. (2019). Factors impairing the postural balance in COPD patients and its influence upon activities of daily living. European Respiratory Journal, 15(1).

[8]Butner, J. D., Martin, G. V., Wang, Z., Corradetti, B., Ferrari, M., Esnaola, N., Chung, C., Hong, D. S., Welsh, J. W., Hasegawa, N., Mittendorf, E. A., Curley, S. A., Chen, S. H., Pan, P. Y., Libutti, S. K., Ganesan, S., Sidman, R. L., Pasqualini, R., Arap, W., ... Cristini, V. (2021). Early prediction of clinical response to checkpoint inhibitor therapy in human solid tumors through mathematical modeling. ELife, 10.

[9]Lawrence, Y. R., Werner-Wasik, M., Dicker, A. P. (2008). Biologically conformal treatment: Biomarkers and functional imaging in radiation oncology. In Future Oncology (Vol. 4, Issue 5).

[10]Dundr, P., Cibula, D., Doležel, M., Fabián, P., Fínek, J., Jirásek, T., Matěj, R., Petruželka, L., Rob, L., Ryška, A., Švajdler, M., Weinberger, V., Zikán, M. (2021).Molecular testing in endometrial carcinoma – joint recommendation of czech oncological society, oncogynecological section of the czech gynecological and obstetrical society, society of radiation oncology, biology and physics, and the society of czech pathologists. Klinicka Onkologie, 34(4).