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Mini-review

Radiogenomics: A systems biology approach to understanding genetic risk factors for radiotherapy toxicity?

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ABSTRACT

Adverse reactions in normal tissue after radiotherapy (RT) limit the dose that can be given to tumour cells. Since 80% of individual variation in clinical response is estimated to be caused by patient-related factors, identifying these factors might allow prediction of patients with increased risk of developing severe reactions. While inactivation of cell renewal is considered a major cause of toxicity in early-reacting normal tissues, complex interactions involving multiple cell types, cytokines, and hypoxia seem important for late reactions. Here, we review 'omics' approaches such as screening of genetic polymorphisms or gene expression analysis, and assess the potential of epigenetic factors, posttranslational modification, signal transduction, and metabolism. Furthermore, functional assays have suggested possible associations with clinical risk of adverse reaction. Pathway analysis incorporating different 'omics' approaches may be more efficient in identifying critical pathways than pathway analysis based on single 'omics' data sets. Integrating these pathways with functional assays may be powerful in identifying multiple subgroups of RT patients characterised by different mechanisms. Thus 'omics' and functional approaches may synergise if they are integrated into radiogenomics 'systems biology' to facilitate the goal of individualised radiotherapy.

Introduction

Radiation therapy (RT) is an important component of modern multimodality tumour therapy and is part of the treatment in approximately 60% of cancer patients treated with curative intent [1]. Although technological advances in the delivery of RT has reduced the volume of normal tissue receiving critical radiation doses, the tumour dose is still limited by adverse effects in adjacent late-reacting normal tissue. Previous studies on RT-induced telangiectasia of the skin suggested that after considering the effects of absorbed dose and dose per fraction, up to 80% of the observed variation in risk was associated with individual patient-related factors [2–4]. The identification of patients' individual susceptibility for the development of adverse effects from RT is an important prerequisite for individualising tumour treatment. Thus the therapeutic window might be widened by increasing the dose to the tumour in patients with relatively radioresistant normal tissue. On the other hand, patients with high risk of developing severe normal-tissue reaction might be candidates for either altered radiotherapy regimens (alternative fractionation schemes, treatment planning, or modalities), changes to surgery (e.g. in breast cancer, mastectomy rather than wide local excision plus RT) or pharmacologic interventions to ameliorate symptoms. Therefore, several approaches to develop a predictive assay for normal-tissue toxicity have been pursued in the past two decades. Here we review the novel high-throughput 'omics' technologies and more classical functional assays and discuss how the two approaches may interact synergistically to facilitate the identification of subgroups with different risks of RT-induced toxicity.

Abbreviations: CFA, colony formation assay; CNV, copy number variation; CFGE, constant-field gel electrophoresis; DSB, double strand break; GWAS, genome-wide association studies; HAT, histone acetyltransferase; HDAC, histone deacetylase; INDELs, insertions and deletions; miRNA, microRNA; NGS, next generation sequencing; PBLs, peripheral blood lymphocytes; PFGE, pulsed-field gel electrophoresis; RILA, radiation-induced lymphocyte apoptosis; RGC, Radiogenomics Consortium; RT, radiotherapy; SF, surviving fraction; SNP, single-nucleotide polymorphism; STAT, standardised total average toxicity.

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Clinical end points, mechanisms and hypotheses

Before reviewing 'omics' approaches and functional assays for radiation-induced normal-tissue reaction, a brief overview of the clinical end points and possible mechanisms is given. This is intended to present the context and basic principles of early and late RT-toxicities based on selected representative examples. For a more detailed discussion of mechanistic aspects, the reader is encouraged to consult comprehensive reviews on the special topics.

In tumour therapy, the aim is to prevent proliferation of tumour cells that will inevitably lead to recurrence of the tumour. Thus the clinical end point of tumour control is closely related to clonogenic inactivation of tumour cells, although the microenvironment and systemic effects may also play a role. Similarly, early reacting normal tissues such as epithelia are characterised by continuous cell renewal with a stem cell niche and a transit-amplifying cell (TAC) compartment forming a differentiation lineage [5,6]. Normally, stem cells divide asymmetrically producing a TAC progenitor cell while retaining a stem cell in its niche. Tissue homeostasis ensures a strict regulation of cell production and loss of terminally differentiated cells by shedding or apoptosis. Thus it is a reasonable assumption that radiation-induced clonogenic cell inactivation is also important for early-reacting tissues [7–9].

An illustrative example is the case of the crypt and villi of the small intestine. Here the stem cell niche consists of 4–6 stem cells protected in the crypts, which produce TACs that form the constantly renewing intestinal lining through amplification and differentiation. If one or more stem cells are lost, the remaining cells temporarily undergo symmetric division until the lost cells are replaced [6,10]. However, if all 4–6 stem cells are lost, the villus will disappear within days because of the high cell turnover. For n stem cells in a crypt, the fraction of inactivated cells after a dose D resulting in a surviving fraction, SF, will be $(1\text{-}SF_D)^n$. Thus, a dose resulting in $SF_D = 0.01$ will inactivate 4–6 stem cells with 94–96% probability. From radiation accidents and experimental animal studies doses $D \geq 10$ Gy to the small intestine are known to be lethal [8]. In addition to targeted inactivation of stem cells, stromal effects may be involved. Thus high single doses of 15 Gy or more have been reported to induce apoptosis of microvascular endothelial cells owing to the release of the second messenger ceramide from membrane sphingo-lipids by acid sphingomyelinase [11]. At doses of approximately 20 Gy and above, newly synthesised ceramide may contribute to apoptosis via induction of ceramide synthase [12,13]. Damage to the capillaries of the small intestine will lead to indirect cell death of stem cells in the crypts, although the role of endothelial apoptosis has been disputed [14,15].

Another experimentally well studied, early reacting system is mouse tongue epithelium. Fractionation studies have demonstrated that stem cells are slowly depleted during the early part of a fractionated schedule. However, when depletion reaches a certain level, i.e. the number of stem cells becomes critical for renewal of the epithelium, some of the stem cells switch from asymmetric to symmetric cell division, allowing cell renewal to match the rate of cell loss during the remaining part of the fractionated schedule [16–18]. The two examples given above show that even for early-reacting tissues with rapid turnover of cells, the role of clonogenic cell inactivation of putative target cells for radiation toxicity is likely to be modified by other biological processes such as a change from asymmetric to symmetric stem-cell division, or between apoptotic pathways. Furthermore, inflammatory reactions stimulated by reactive oxygen species (ROS) and debris from cell death, and mediated by cytokines and immune cells, play an important role in early reaction [19]. Immune cells in turn produce ROS that may produce further oxidative DNA damage in the normal tissue, even in cells outside the irradiated region [20,21].

Based on the role of inflammation and immune cells in the radiation response of tumour and normal tissues, a bioinformatics approach was recently used to identify a molecular network of 24 genes with differential expression common to cancer and healthy tissues [22]. Prominent genes coded for members of the NF-κB family and other transcription factors involved in transcriptional activation of cytokines, and for cytokine receptors, chemokines, cytokines, growth factors, signalling molecules, and cell adhesion molecules involved in the recruitment of immune cells to injured tissues. Genes differentially regulated by irradiation of healthy tissue included radiation response and cell cycle genes TP53, CDKN1A, and CCND1, several genes involved in cytokine expression and signal transduction, but also pro- and anti-apoptotic genes, and genes coding for collagen type 1 and 3 chains. Most of the genes were identified by irradiation of human peripheral blood or brain tissue of experimental animals, and the expression profile is consistent with the role of cytokines and immune cells in normal-tissue reaction, and the apoptotic response of lymphocytes to moderate doses of ionising radiation. However, NF-κB is not limited to blood cells and the brain. Thus radiation-induced expression, activation, or translocation, of NF-κB is involved in controlling numerous pro-inflammatory genes, chemokines, chemokine receptors, and cell adhesion molecules, in several different cells and tissues [23].

Late reacting tissues rely much less on cell renewal than early reacting tissue. In many cases, fibrotic changes in connective tissue or organs are involved. Fibroblasts do not readily undergo apoptosis after irradiation but are permanently arrested and continue to be metabolically active for extended periods of time (months and years) [24,25]. Although irradiated fibroblasts are frequently referred to as being senescent, premature terminal differentiation is a more appropriate term as they synthesise increased amounts of extracellular matrix proteins, and show decreased proteolytic activity with downregulated expression of matrix metalloproteases (MMPs) and upregulated expression of tissue inhibitors of MMPs (TIMP) [26–30]. Furthermore, some cells differentiate to contractile myofibroblasts expressing α-smooth muscle actin [31,32]. Although true mesenchymal stem cells may only be present in the bone marrow, a mixture of progenitor cells in the TAC compartment, including early stages with high residual capacity for proliferation, can be isolated by outgrowth from skin samples. Because differentiation of progenitor fibroblasts occurs along a long TAC differentiation lineage with exponential expansion, division of rare stem cells or early progenitor cells is sufficient to maintain homeostasis of the tissue [33].

Various studies suggest that different normal-tissue end points do not always correlate closely within individual RT patients. Thus neither erythema nor subdermal fibrosis correlated with telangiectasia of the skin in breast cancer RT patients [3,34]. However, a more recent, larger study showed a significantly increased risk of fibrosis in breast RT patients with telangiectasia, although overall, the risk of developing fibrosis was much lower than that of telangiectasia [35]. In the RAPPER ("Radiogenomics: Assessment of Polymorphisms for Predicting the Effects of Radiotherapy") study, which comprises 778 breast RT patients enrolled in the Cambridge breast IMRT (intensity-modulated RT) trial, correlation coefficients were generally low, although significant correlations were found between several end points (e.g. between breast shrinkage and six other end points, as well as between breast induration, edema, and telangiectasia) but not between breast induration and telangiectasia [36]. The lack of close correlations between different end points, in particular between radiation-induced fibrosis and telangiectasia, supports the view that there are differences in the mechanisms underlying the pathogenesis of different end points. Thus fibrotic late reactions have been hypothesised to result from complex interactions between different cell types involving inflam-