Cancer Letters 382 (2016) 127–136

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Mini-review

Radiogenomics – current status, challenges and future directions

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ARTICLE INFO

Keywords:
Normal tissue toxicity
Radiosensitivity
Predictive assay
SNP
Complex trait
Expression quantitative trait loci

ABSTRACT

Radiogenomics designates a scientific field that addresses possible associations between genetic germline alterations and normal tissue toxicity after radiotherapy. The ultimate aim of this research is to establish a gene-based predictive test for normal tissue radiosensitivity. During the last 5 years, substantial progress has been achieved in this field. Several compelling associations for SNPs have been demonstrated in large candidate gene studies as well as genome wide association studies. These findings shed new light on radiobiology and expand our understanding of the processes that lead to side effects after radiotherapy. Despite this, certain fundamental challenges still relate to genomic approaches. Based on the latest insights into complex trait genetics and molecular genetics, we provide an analysis of these challenges and propose putative strategies to further advance the field. These strategies include 'big data approaches' and collaborative research within international consortia. Furthermore, research that combines the study of radiation-induced gene expression and genome-wide SNP genotype may discover genetic alterations that regulate the biological response to ionizing radiation. Thus, such integrative approaches may lead to genetic alterations that affect risk of normal tissue toxicity.

Background

Radiotherapy represents a very important treatment modality in the management of cancer, either alone or as part of combined modality therapy. Approximately 50% of all cancer patients will receive radiotherapy at some time in their illness [1]. Compared to surgery, radiotherapy has the advantage of being potentially organ and function preserving. Nevertheless, it may lead to severe acute or late side effects. Apart from causing pain and distress during treatment, acute normal tissue reactions can result in treatment interruptions or may progress into lasting 'consequential late damage'. Late normal tissue reactions may cause chronic disability and compromise long-term quality of life. Consequently, radiation induced normal tissue reactions represent a dose limiting factor in radiotherapy. Typically, treatment regimens are designed to ensure that the risk of severe late effects does not exceed 5–10% [2]. This means that a small fraction of radiosensitive patients limits the dose that can be given to the entire patient population, although the majority of patients could potentially tolerate a higher dose. It is estimated that more than 80% of the variability in normal tissue radiosensitivity can be attributed to patient-related factors rather than stochastic effects [3]. If this variability could be taken into account in the treatment-planning phase, the therapeutic strategy could be individualized accordingly [4]. Patients being relatively sensitive to the effects of ionizing radiation could (when possible) be offered a treatment strategy that does not include radiotherapy whereas the resistant patients could be dose escalated to some extent. Using data for late skin toxicity, it is estimated that it might be possible to dose escalate the most resistant 40% of the patients by almost 20% [2]. Due to a relatively steep dose–response relationship for tumour control, such dose escalation would potentially translate into a substantial gain in tumour control probability. Therefore, the ability to predict normal tissue complication risk prior to therapy has been a long sought goal in radiobiology. This goal is further accentuated by the recent advent of particle therapy. This treatment modality has the potential to spare the normal tissues but is so far only accessible for a limited proportion of the cancer patients. Thus, careful patient selection will be of particular importance in this context.

Radiogenomics – the vision

Radiogenomics designates a scientific field that addresses possible associations between genetic germline variation and normal tissue toxicity after radiotherapy [4]. The research has two main purposes: the ultimate aim is to establish a gene-based predictive test for normal tissue radiosensitivity that could be used to support clinical decision-making. Equally important, the research into genotype–phenotype relationships in radiobiology has the potential to broaden our understanding of the mechanisms that lead to radiation-induced

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http://dx.doi.org/10.1016/j.canlet.2016.01.035
0304-3835/

normal tissue damage. Thus, the research may uncover pathways and processes that could serve as targets for pharmacological strategies to prevent or mitigate side effects after radiotherapy.

The history

In the late 1990s increasing interest was taken in the hypothesis that normal tissue radiosensitivity is under genetic control and that normal tissue complication risk could be predicted from genetic analysis [2]. This concept received support from the observation that patients suffering from certain rare genetic syndromes such as ataxia telangiectasia, Blooms syndrome, Fanconi's anaemia and Nijmegen breakage syndrome experience devastating normal tissue reactions if treated with radiotherapy [5]. All these syndromes are related to mutations in genes involved in detection of DNA damage and initiation of DNA repair. Nevertheless, these syndromes characterized by Mendelian inheritance are extremely rare and probably of little relevance when addressing the average cancer patients [2]. It was hypothesized that heterozygous carriers of pathogenic (truncating) mutations in *ATM* and *BRCA1* and *BRCA2* could constitute a radiosensitive subpopulation. This assumption received support from the observation that cells from heterozygous carriers of *ATM* mutations exhibited cellular radiosensitivity that was intermediate compared to ataxia telangiectasia patients and normal controls. Nevertheless, a number of relatively small studies did not provide any indications that these genetic alterations are overrepresented among patients with excessive normal tissue reactions nor that carriers of truncating *ATM* or *BRCA* mutations exhibit a higher normal tissue complication risk than the average patient. Similarly, no obvious association was found between clinical normal tissue radiosensitivity and mutations in other DNA repair genes like *RAD50*, *RAD21*, *NBN* or *MRE11A* (reviewed in [6]). In the interpretation of these results, the limited statistical power of the studies should be taken into account.

Basic hypotheses

At the turn of the millennium, substantial efforts were made to unravel the genetics underlying a variety of different biomedical phenotypes. This impetus in research activity was to a large extent driven by a rapid progress in genotyping technology and the sequencing of the human genome. Because of these advances, a more comprehensive hypothesis about the genetics of normal tissue radiosensitivity could be formulated [6]. This hypothesis is encompassed in the following three paragraphs:

(1) *Normal tissue radiosensitivity is as a complex trait dependent on the combined influence of sequence alteration in several genes.*

The mere observation that normal tissue radiosensitivity is characterized by a continuous spectrum rather than falling into distinct categories is per se indicative of a polygenic inheritance. The biological response to irradiation is presumably very complex and involves a multitude of different pathways and genes. Given the widespread existence of sequence alterations in the genome, numerous loci with potential impact on clinical radiosensitivity are likely to exist [6].

(2) *Single nucleotide polymorphisms may make up a proportion of the genetics underlying differences in clinical normal tissue radiosensitivity.*

One of the important insights provided by the sequencing of the human genome was that single nucleotide polymorphisms (SNPs) are widespread throughout the genome. A SNP is defined as a single base substitution in which the least common allele has an abundance of 1% or more in general population. It is estimated that around 11 million SNPs exist throughout the human genome and that SNPs account for approximately 90% of the inter-individual sequence variation within human populations. SNPs in coding regions may alter protein function whereas SNPs in regulatory regions may affect gene expression/protein synthesis rate. Thus, SNPs have the potential to affect various phenotypes including normal tissue radiosensitivity. This assumption represents the cornerstone of the so called 'common disease – common variant' hypothesis according to which common sequence alterations (such as SNPs) make up a large proportion of the inherited predisposition to various common conditions [7]. However, it also seems likely that rare sequence alterations may affect normal tissue radiosensitivity [8] (this aspect is further discussed later in this paper).

(3) *Some genetic alterations are expressed selectively through certain types of normal tissue reactions, whereas others exhibit a 'global' impact on radiosensitivity.*

Clinical studies addressing normal tissue radiosensitivity have indicated that the risk of different types of adverse reactions after radiotherapy is not strongly associated with each other when adjustments are made for differences in treatment characteristics and length of follow-up [9]. This observation indicates that some genetic alterations have to be expressed selectively through certain types of normal tissue reactions. However, patients suffering from one of the aforementioned 'radiosensitive syndromes' appear to experience a general enhancement of clinical radiosensitivity apparently affecting both acute and late morbidities [5]. Based on this, it seems likely that some genetic alterations affect clinical radiosensitivity in a generalized manner whereas others exhibit a differential expression through different types of normal tissue reactions [6]. From a mechanistic perspective, these assumptions seem plausible. The molecular pathways (and genes) underlying different types of normal tissue reactions probably differ substantially from each other. This allows for a differential impact of some genetic alterations across different types of normal tissue toxicity.

Candidate gene studies

A total of 128 candidate gene studies have been carried out in normal tissue radiobiology (Fig. 1). As mentioned earlier, a few rather small studies have addressed the impact of truncating *ATM* and *BRCA* mutations upon normal tissue radiosensitivity. Apart from this, the efforts to unravel the genetics of normal tissue radiosensitivity have primarily focused on SNPs. With a median sample size of less than 150 patients, most of the studies were very small. Only ten studies included more than 500 patients and only five studies included more than a thousand patients. These studies typically investigated SNPs in genes involved in processes such as detection of DNA damage (i.e. *ATM*), DNA repair (i.e. *XRCC1*, *XRCC3* and *APEX*) tissue remodelling (i.e. *TGFB1* and *TIMP*) and scavenging of reactive oxygen species (i.e. *SOD2* and *GSTP1*). More than 100 different genes were investigated as part of this research. Around two thirds of these studies have reported significant associations [10]. Nevertheless, the findings were often inconsistent and independent replication of previously reported associations rarely took place. In hindsight, it seems obvious that the vast majority of these studies have suffered from methodological shortcomings. First, most of the studies were severely underpowered to detect the small effect sizes that are usually found for SNPs. Furthermore, the studies often had unattended multiple testing problems [8,10]. Nevertheless, a few candidate gene studies have actually reported compelling SNP associations. These studies included more than a thousand patients and involved independent replication cohorts. A study published