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Overview

Radiogenomics in the Era of Advanced Radiotherapy

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Abstract

Most radiogenomics studies investigate how genetic variation can help to explain the differences in early and late radiotherapy toxicity between individuals. The field of radiogenomics in photon beam therapy has grown rapidly in recent years, carving out a unique translational discipline, which has progressed from candidate gene studies to larger scale genome-wide association studies, meta-analyses and now prospective validation studies. Genotyping is increasingly sophisticated and affordable, and whole-genome sequencing may soon become readily available as a diagnostic tool in the clinic. The ultimate aim of radiogenomics research is to tailor treatment to the individual with a test based on a combination of treatment, clinical and genetic factors. This personalisation would allow the greatest tumour control while minimising acute and long-term toxicity. Here we discuss the evolution of the field of radiogenomics with reference to the most recent developments and challenges.

Key words: Genome-wide association studies; radiogenomics; radiotherapy toxicity

Genetic Variation

Although it has been estimated that up to 80% of the differences in toxicity can be explained by intrinsic patient variation [1], there are also other factors to take into consideration, which influence the likelihood of developing radiotherapy side-effects [2]. These include radiation dose, technique, fractionation, other concomitant treatments, age, smoking status and comorbidities. Radiosensitivity is a polygenic trait affected by multiple genes and biological pathways.

There are key principles of genetic variation that underpin the study of radiogenomics. Single nucleotide polymorphisms (SNPs) are DNA sequence variations that occur when a single nucleotide within a gene is altered. A typical genome differs from the reference human genome at 4.1 million to 5.0 million sites; 99.9% of variants consist of SNPs and short insertions or deletions of nucleotides [3] The minor allele frequency (MAF) of a SNP is the frequency of the less common nucleotide variant. Multiple SNPs are inherited together in haplotype blocks, which occur due to recombination hotspots during crossover in meiosis [2]. A 'tag' SNP represents the haplotype and predicts for the presence of several other SNPs in the block (Figure 1). These SNPs are said to be in linkage disequilibrium.

Other forms of genetic variation exist. Structural variants include deletions, insertions, duplications and inversions. Copy number variations (CNVs) are large sections of the genome in specific chromosomal regions that are repeated and the number of repeats varies between individuals. CNVs may alter the levels of gene expression; they are less numerous than SNPs but, because they span large parts of the genome, account for 5–10% of the human genome. These forms of genetic variation occur in both coding and non-coding areas of the genome. Less than 3% of the human genome is DNA that is transcribed and translated into proteins; the remainder has important regulatory function and codes for transcription enhancers and silencers,

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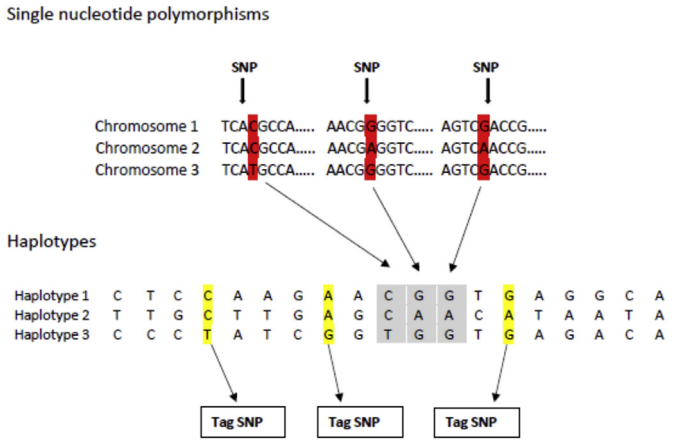

Fig 1. Haplotype blocks and the principles of linkage disequilibrium. Three single nucleotide polymorphisms (SNPs) in three different chromosomes are shown in linkage disequilibrium and lying within the same haplotype block. Tag SNPs can be used to screen a length of DNA, predicting the presence of other SNPs with which they are in linkage disequilibrium.

regulators of gene methylation, modifiers of chromatin structure and non-coding RNAs with regulatory function. Any of these may play a part in determining an individual's radiosensitivity.

Radiosensitivity Syndromes

The discovery of radiosensitivity syndromes provided the first evidence for an association between genetics and the risk of radiotherapy toxicity. These syndromes are characterised by the Mendelian inheritance of rare germline mutations in specific genes involved in DNA repair and damage response, leading to genomic instability and cancer predisposition. Ataxia telangiectasia was the first syndrome described. Ataxia telangiectasia is a rare, neurodegenerative, autosomal recessive disease, causing severe disability and a hypersensitivity to ionising radiation. Patients experience severe acute toxicity during radiotherapy [4–6] and their cells show reduced clonogenicity and increased chromosomal damage after exposure to ionising radiation [7,8]. Ataxia telangiectasia was subsequently linked to truncating mutations in both copies of the ataxia telangiectasia (*ATM*) gene. Radiosensitivity syndromes are rare and probably of little relevance when assessing radiosensitivity in most radiotherapy patients. They also seem to affect global radiosensitivity, including early, late and long-term carcinogenic effects, whereas other types of genetic variation may be more normal tissue-type specific.

Candidate Gene Studies

The first radiogenomics studies were small and adopted the candidate gene approach. Candidate genes are known or thought to be involved in the development of radiotherapy toxicity, such as those involved in DNA repair (*ATM*, *BRCA1*, *BRCA2*, *TP53*), anti-oxidation (*SOD2*) and cytokine signalling (*TBFB1*). The hypothesis underlying studies of candidate genes is that, as rare homozygote mutations are known to affect radiosensitivity, common SNPs would have smaller effects on our phenotype of interest. The problem with the first candidate gene studies is illustrated using transforming growth factor beta (TGF-β1). Early studies suggested a relationship between elevated serum TGF-β1 levels and the development of fibrosis in lung and breast cancer patients [9,10]. However, radiogenomics studies found no consistent relationship between common genetic variants in the *TGFB1* gene and radiotherapy toxicity [11,12]. Barnett *et al.* [12] highlighted the limitations of early radiogenomic studies when they failed to validate any previously reported associations in a study of 1613 patients. Candidate gene studies have mainly been underpowered to detect the small effect sizes that are expected for SNPs, and many studies failed to account for multiple testing [13]. Of course, the main drawback of candidate gene studies is that they require some *a priori* knowledge of gene function, which means that unknown genes involved in the phenotype are missed. Polygenic traits involve a combination of rare variants with large effects, moderately common variants with moderate effects and hundreds of common variants with small effects. Any focus on candidate genes in radiogenomics, therefore, overlooks the polygenic nature of radiosensitivity.

International Radiogenomics Consortium

The international Radiogenomics Consortium (RGC) was established in 2009 to address these issues [14]. The consortium comprises 219 members, 129 institutions and 32 countries, and provides a collaborative link between groups performing genotyping studies. The RGC is a route for sharing expertise, developing methodology and achieving large, sufficiently powered studies. Multivariable analysis is recommended to account for other factors that influence radiation toxicity, and so increase the chance of identifying a true genetic association. The RGC developed and recommends use of the STROGAR guidelines: Strengthening the Reporting of Genetic Association Studies in Radiogenomics [15].

The first work of the RGC was focused on individual SNPs from candidate genes, but on a much larger scale than in early published studies. A meta-analysis of individual patient data investigated the association between *TGFB1* SNP rs1800469 and the development of late radiotherapy toxicity in 2782 breast cancer patients. No association was found with risk of subcutaneous fibrosis or overall toxicity, but the meta-analysis did show successful collaboration within the RGC [16]. Subsequent large collaborative studies identified validated SNPs. Analyses of combined breast cancer cohorts showed associations between SNPs near or in *TNF* ($n = 2036$) [17] and *XRCC1* ($n = 753$) [18] and the risk of radiotherapy toxicity. A meta-analysis of 5456 breast and prostate cancer patients found an association between a minor allele of SNP rs1801516 in *ATM* and increased early

and late toxicity (odds ratio 1.2–1.5) following radiotherapy [19]. Thus, adequately powered candidate gene studies can make a useful contribution, but still require a hypothesis based on prior knowledge of radiation biology.

Genome-wide Association Studies

The field of radiogenomics progressed to genome-wide association studies (GWAS) to allow hypothesis-free exploratory research. The principle of a GWAS is to genotype between 300 000 and 1 000 000 tag SNPs, which represent most of the known common variation within the genome. Other SNPs are imputed using the principles of linkage disequilibrium. The 1000 Genomes project involved the analysis of over 1000 patients' genomes across 14 countries, providing a validated haplotype map of 38 million SNPs, 1.4 million short insertions and deletions and more than 14 000 larger deletions [3]. Data from this project are used to estimate the genotypes of variants between those that were included on the SNP array, i.e. those that were directly genotyped. This has facilitated meta-analysis of GWAS, even if the individual GWAS cohorts used different DNA chips. It also means that, although a few thousand SNPs might be directly genotyped, several million are imputed in a process known as fine mapping and used to identify relationships between genotype and phenotype. One technique for fine mapping is to identify 1 Mb regions surrounding each significant independent association, and then use a reference panel and imputation algorithm to re-impute the genotypes [36].

GWAS analyses obviously involve the problem of multiple testing; a Bonferroni correction is required and $P < 5 \times 10^{-8}$ is considered significant. The likelihood of a statistically significant association being detected is affected by the imputation score (r^2, the higher the value the stronger the linkage disequilibrium and the likelihood of identifying a causal variant). The power to detect an association is also influenced by the MAF and the effect size of the SNP, the study size and the prevalence of the phenotype. Some statistically significant associations will probably be false positives and it is now recommended that the Bayesian false discovery probability (BFDP) is also calculated [20]. The BFDP is particularly relevant to the interpretation of genetic studies as it is a means of assessing the probability of the hypothesis given the data. The aim of the BFDP is to prevent over-interpretation of statistically significant findings as defined by the *P* value, that are not likely to signify true associations. The BFDP therefore takes into account the power of a study to detect an association to control the number of false-positive findings and assess the noteworthiness of the results.

The advantage of GWAS is that they study all SNPs, including those in regulatory regions and areas of the genome whose function is not yet fully understood. The underlying assumption of GWAS is that SNPs in many genes contribute to genetic variation in the population, but they make no assumptions about which SNPs or genes are important in the relevant biological process. GWAS of traits and diseases therefore look for common genetic variants, each with small effects. Most genetic variants surveyed through GWAS have a MAF >1% and the typical effect size of each SNP is small, with relative risk or odds ratios of typically <1.2. Significant associations between the tag or imputed SNP and the phenotype indicate the presence of nearby causal variant SNPs, but do not identify the causal variant itself. Increasingly, there is recognition of the importance of understanding the regulation of gene expression and the role of SNPs and this can be evaluated in the expression quantitative trait locus analysis in GWASs. These analyses focus on evaluation of the regulatory control exerted by nearby (cis) and distant (trans) loci on the expression of genes influencing a phenotype [21].

Radiogenomics Genome-wide Association Studies

The first GWAS looking at SNPs in relation to radiotherapy toxicity was a small study of 79 African American prostate cancer patients. The study identified a possible SNP associated with erectile dysfunction located within a gene that encoded a protein involved in male gonad development and function [22]. Initial GWAS were small due to the high cost of genotyping and mostly involved a staged approach with an initial GWAS followed by validation of the most significant SNPs in independent cohorts. The first two-stage study ($n = 465$) identified a set of SNPs associated with the development of erectile dysfunction following radiotherapy for prostate cancer [23]. In a further GWAS, a region on chromosome 9p21.2 containing eight SNPs showed an association with urinary toxicity in a cohort of 723 prostate radiotherapy patients [24]. Novel genetic markers of rectal bleeding following prostate radiotherapy have also been found in a two-stage discovery and replication GWAS ($n = 187$ cases and 962 controls) [25]. This identified two SNPs that tag a locus on chromosome 11q14.3. The UK RAPPER group carried out a larger first-stage GWAS in 1850 patients with breast ($n = 1217$) or prostate ($n = 633$) cancer. The GWAS showed that associations in breast cancer patients were different to those identified in prostate cancer patients [26]. The second-stage validation of the top SNPs involved 355 breast and 1378 prostate cancer patients. The study provided the best evidence that there are many associations to be found and that further larger studies would be worthwhile. The strongest associations were for individual toxicity end points rather than an overall measure of toxicity.

Fachal *et al.* [27] identified a susceptibility locus for late urinary and rectal toxicity at *TANC1*, which has a role in regenerating damaged muscle. The first phase of this large study included 741 men with prostate cancer (417 with late toxicity) from the Spanish RADIOGEN study; replication was carried out in 579 men from the UK RAPPER study and 270 men from the US Gene-Pare Study, allowing validation with a multistage approach. SNP rs264662 was associated with the level of TANC1 mRNA expression using expression quantitative trait locus analysis. This GWAS showed that

new loci of interest can be found and validated effectively. The first GWAS also showed that the common variants associated with late effects seemed to be in or near genes associated with normal tissue function. Of course, we are not certain which is or are the causal SNP(s) affecting biological function, and some SNPs may be missed if not identified in the early, smaller scale phase.

Most radiogenomics studies focus on the late effects of radiotherapy – those occurring 2–5 years after treatment. Second cancer induction is a very late effect. Best *et al.* [28] identified variants at 6q21 that implicated *PRDM1* in the aetiology of radiotherapy-induced second malignancies after treatment for Hodgkin's lymphoma. Morton *et al.* [29] conducted a GWAS of breast cancer in female survivors of childhood cancer that included 207 women who developed breast cancer and 2774 who did not. A locus on 1q41 reached genome-wide significance for an association with breast cancer risk (rs4342822, near *PROX1I).* These studies provide strong evidence that common genetic variants modify the effect of radiation exposure on second cancer induction.

Genome-wide Association Studies Meta-Analyses

GWAS meta-analyses have further increased the size of the cohorts studied. The use of a staged approach in the first GWAS was driven by the costs of genotyping. As these costs have fallen, a second-stage genotyping of a smaller number of SNPs (the 'top hits') is not needed. Statistical power improves by maximising the number of patients analysed in the discovery phase. There is in-built validation due to the size of the analysed population. Experience from GWAS for other traits and diseases shows that, with successive single-stage meta-analyses, as the overall cohort size increases some SNPs will drop out (false positives) and others will become more significant (validated SNPs). The first meta-analysis of radiogenomics GWAS studies used a fixed effects meta-analysis [30]; 1564 men with prostate cancer undergoing radiotherapy were included. Two end point-specific and one SNP associated with overall toxicity were identified. Rs17599026 was associated with increased urinary frequency and rs7720298 was associated with decreased urinary stream. These SNPs lie in genes that are expressed in tissues that are adversely affected by radiotherapy to the pelvis. The study successfully showed that heterogeneous groups of patients recruited in different trials and countries could be co-analysed effectively. Multivariable analysis is required to reduce the effect of the more heterogeneous aspects of the different studies in terms of radiotherapy fractionation regimens, additional treatments (e.g. surgery, hormones and chemotherapy), patient characteristics and the mean biological effective dose.

A second meta-analysis was completed in 2018, combining data from the first meta-analysis with new data generated via the OncoArray Network. The RGC collaborated with the OncoArray Network, which assembled more than 400 000 samples from existing studies and several biobanks. Genotyping involved the Illumina Infinium OncoArray-500K BeadChip, currently the most widely used in radiogenomics GWAS. The OncoArray was developed for researching genetic variants associated with the risk of developing breast, colorectal, lung, ovarian and prostate cancers. The array has a genome-wide backbone of about 275 000 tag SNPs, additional SNPs associated with the incidence of these cancers, plus SNPs covering ancestry, quantitative traits, pharmacogenetics and fine-mapping of common cancer susceptibility loci. The top 1000 hits from the RAPPER prostate and breast GWAS were also included. The latest meta-analysis included 3874 RGC prostate patients and identified new associations with increased risk of late toxicity.

Future Directions

Studies to date highlight that continued efforts in radiogenomics are worthwhile and should be pursued. At the tumour level there is a need to increase the cancers studied to cover all those where radiotherapy has a role in potentially curative treatment. Most published studies have focused on patients with prostate or breast malignancies. The EU-funded REQUITE project generated a lung cohort [31], which was OncoArray genotyped together with three other cohorts ($n = 909$). The RGC head and neck group are working towards a meta-analysis of about 10 000 patients. The RAPPER group recently genotyped cohorts of sarcoma and rectal cancers. There are many other cancers where biorepositories should be built, such as paediatric and central nervous system.

At a clinical level, the increasing use of charged particle therapy including protons and carbon ions presents a new challenge. To date, radiogenomics studies have focused on photon therapy. Different types of radiation have different biological effects and different patterns of DNA damage. Although some genetic variants will probably affect toxicity and second malignancy risks for both photons and charged particles, it is plausible that others may be specific to the radiation modality [32]. The end points and toxicities will vary and may require longer follow-up (especially for second malignancies). The recent opening of a National Health Service proton beam therapy centre in Manchester presents an opportunity to collect a blood sample from all patients treated; efforts are underway to try to make this happen. Of course, protons are not as widely used as photons and there may be a difficulty in achieving large numbers for radiogenomics studies, which underlines the need for international collaboration.

At a genetic level, there is potential to evaluate other types of genetic variation, such as nucleotide insertions, deletions and CNVs, which can all be evaluated in GWAS. Genetic analysis is becoming quicker, more cost-effective and more readily available, with high-throughput, base-pair resolution of whole-genome sequencing becoming more focused and cost-effective in the form of targeted disease-associated next-generation sequencing enabling the study of rarer genetic variants [33].

At an application level, there is a need to generate and validate polygenic risk scores that are part of clinical models/nomograms. The next stage for SNPs identified in the above studies is validation of risk models incorporating genetic information. The REQUITE study collected standardised data from 26 hospitals in eight countries. The centralised database, linked to a centralised biorepository, includes data on patients (e.g. age, body mass index, comorbidities), treatment (e.g. radiotherapy regimen, additional treatments received) and outcomes (e.g. patient-reported outcome measures, physician-reported toxicity, survival). The centralised database also includes radiotherapy physics files (DICOM, DVH), breast photographs (breast cancer patients only) and genotyping data [34]. The study collected data for about 4500 patients with prostate, breast or lung cancer. An additional 543 lung cancer patients were genotyped and the data added to the database. The resource was established by members of the RGC so that risk prediction models incorporating genetic information could be validated in a multicentre setting. REQUITE collaborators also designed possible future trials that could use validated models to individualise treatment with the goal of reducing the number of patients suffering with the long-term consequences of radiotherapy.

Challenges

The field of radiogenomics has clearly progressed rapidly and SNP genotyping is now very affordable, but there remain challenges to address. Multicentre clinical trials and individual centre studies enabled the collection of cohorts with stringent radiotherapy standards and standardised toxicity assessment, but this is more challenging in routine 'real-world' clinical practice, where any test would be applied. The success of REQUITE in collecting real-world patients was underpinned by funding to collect large amounts of data. Thousands of patients undergo radiotherapy every year, but the collection of toxicity data is sparse. There is a need for centres and funders to invest in the routine collection of toxicity data.

A second challenge is to ensure that radiogenomics studies include patients with multiple ethnicities. Current GWASs involve an initial step to exclude non-Caucasian ethnicities to reduce potential confounding by population structure (relatedness), effects that reduce the ability to identify risk SNPs. The most recent RGC meta-analysis included cohorts of Japanese patients that were analysed separately to investigate whether SNPs identified in Caucasians were also involved in the risk of toxicity in those with Japanese ancestry ([AQ2]Kerns *et al.*, submitted). More effort is required to support biorepositories focusing on non-Caucasian ethnicities and to validate methods used to co-analyse multiple ethnicities. Cook and Morris [35] evaluated a framework that allows for population structure when investigating associations between genetic variants and complex traits. Future GWASs will be able to build on these findings and expand the generalisability of the results.

To date, the large collaborative studies focused mainly on late toxicity, but future studies should also include the assessment of acute toxicity, to ensure that potential increases in dose do not lead to intolerable early or consequential late effects. It is likely that different metabolic processes are responsible and so genetic variation may affect early and late effects differentially. It would also be useful to understand the genetic variation underlying spinal cord damage, which often limits dose and tolerance to retreatment.

It is also currently unclear what the effect is of genetic risks of toxicity to concurrent treatments, such as cisplatin, cetuximab, nimorazole and immunotherapy, and how these will modulate radiation dose–response relationships. The identification of risk SNPs in pharmacogenomics studies is relevant in radiogenomics with the increasing use of multimodality treatments. Retrospective analysis of trials comparing radiotherapy alone or with radiosensitisers will be useful but must be underpinned by the routine collection of blood samples.

Conclusion

The era of advanced multimodality radiotherapy requires the development of approaches for tailoring treatments to the individual. Radiogenomics can contribute by identifying sufficient genetic variants associated with a patient's risk of developing radiotherapy toxicity. Although the future would involve a single genetic test of multiple variants, we currently expect polygenic risk scores to be calculated using different sets of end point-specific genetic variants that are then incorporated into end point-specific clinical models/nomograms. These models/nomograms could be used to safely deliver higher doses to the tumour volume in low-risk patients and avoidance strategies in those with a high risk of radiotherapy toxicity. A predictive test could also enable decisions about which treatment to offer a patient (e.g. surgery rather than radiotherapy), the ordering of treatments and early symptom control. Patients with a low risk of long-term toxicity might also benefit from an increased scope for re-irradiation if required.

Improved understanding of radiogenomics may help to understand the biological processes underlying radiotherapy toxicity, which may lead to better approaches for pharmacological manipulation in the future. However, understanding the functional effect of a SNP may not always be possible and is not necessary for risk prediction. Progress in radiogenomics has not only identified multiple SNPs for further validation, but also addressed methodology issues around study design and analysis to maximise the quality of research carried out. Further research will expand the number of genetic variants associated with radiosensitivity, validate risk prediction models and underpin progress in our ability to personalise radiotherapy.

Conflict of interest

The authors declare no conflict of interest.

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