Outline

1. Patient variability and the need for predictive assays
2. Profiles based on DNA variation to predict normal tissue response
3. RNA expression profiles to predict tumour response

Inter-patient variability

- Tumour control (TCP)
- Normal tissue damage (NTCP)
- Radiation dose (Gy)

Predictive assays aim to individualise dose prescriptions and improve the therapeutic ratio

- Tumour radiosensitivity measured in 156 patients with cervix tumours prior to radiotherapy predicts for the probability of cancer-specific survival

Molecular profiling – why?

- Developing molecular signatures to predict how patients will respond to radiation
- Rationale: to increase the individualisation of radiotherapy and decrease cancer mortality and morbidity
- Improve the therapeutic ratio based on biological optimisation
The Genomic Era

- 2001 - draft sequence published
- April 2003 - full sequence available
- Exact number of genes uncertain
- October 2004: estimated number of human protein-coding genes reduced from 35,000 to 20,000-25,000
- Caenorhabditis elegans (earthworm) has ~19,000 genes
- Oryza sativa (rice) has 32,000-56,000 functional genes

The Genomic Revolution

- New high throughput technologies and capabilities for analysis of a large number of genes/whole genome
- Analyses can be at the DNA (genome), RNA (transcriptome) or protein (proteome) level
- Future personalised treatment based on molecular profiling

The biological factors known to underlie variation in patient response to radiotherapy can be exploited to develop gene/gene expression profiles

- Normal tissue radiosensitivity
- Tumour radiosensitivity
- Tumour hypoxia
- Tumour proliferation

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DNA

Looking at genetic variation to predict normal tissue toxicity

- Individuals vary in their intrinsic sensitivity to radiation
- Normal tissue radiosensitivity is inherited
- Some studies showed that intrinsic normal cell radiosensitivity predicted normal tissue response

Some studies showed a relationship between cellular radiosensitivity measured in vitro and the probability of a patient developing radiation toxicity

Prospective study showing pre-treatment lymphocyte radiosensitivity (SF2) is a significant independent prognostic factor for the likelihood of developing late toxicity following radiotherapy

n = 83 cervix cancer patients

West et al 1998, 2001
Current understanding of the genetic basis for individual sensitivity to radiation

- **Variations** in many genes are likely to underlie patient difference in normal tissue response to radiotherapy (complex polygenic trait)
- DNA damage recognition and repair, cell cycle control, apoptosis, inflammatory response, pro-fibrotic response
- Developing radiosensitivity associated profiles based on genetic variation is the way forward

Profiling radiosensitivity: mutations vs polymorphisms

- DNA sequence variations are described as mutations or polymorphisms
- Mutation - any change in a DNA sequence away from normal that is a rare and abnormal variant
- Any new sequence variant will start off as a rare mutation
- Polymorphism - a common DNA sequence variation with an arbitrary cut-off of 1% for the least common allele
- Polymorphic sequence variants influence characteristics such as height and hair colour

Single nucleotide polymorphisms (SNPs)

- For example a SNP might change the DNA sequence AAAGCTTA to ATGGCTAA
- The availability of a reference sequence of the genome provides the basis for studying genetic variation, particularly SNPs
- SNPs make up ~90% of all human genetic variation & occur every ~300 bases along the 3-billion-base human genome
- ~7 million SNPs in the human genome

Prediction of normal tissue response from SNPs in candidate genes. In a study of 41 patients with breast cancer, those with many risk alleles were radiosensitive.

<table>
<thead>
<tr>
<th>Candidate genes: TGFB1, SOD2, XRCC1, XRCC3, APEX</th>
<th>ED50: the radiation dose which would on average be expected to cause a certain normal tissue effect in 50% of the treatment fields</th>
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<tbody>
<tr>
<td>n = 41</td>
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Increasing number of studies looking at genetic variation in relationship to radiotherapy normal tissue response

<table>
<thead>
<tr>
<th>Reference</th>
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<td>ATM codon 1853 Asn/Asp &amp; Asn/Asn genotypes associated with grade 3 fibrosis in breast pts</td>
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<td>De Ruyck 2006</td>
<td>TGFβ1</td>
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<td>Homozygous variant TGFbeta1 -1.552delAGG, -509TT, and 10Pro genotypes associated with severe clinical radiosensitivity after gynecologic RT</td>
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<td>Damaraju 2006</td>
<td>LIG4, ERCC2, CYP2D6</td>
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<tr>
<td>Kornguth 2005</td>
<td>ERCC4</td>
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<td>ERCC4 T2505C (codon 835 Ser/Ser (silent)) associated with reduced need for long-term feeding tube following radiotherapy</td>
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<td>Chang-Claude 2005</td>
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<td>XRCC1 399Gln &amp; APE1 148Glu alleles associated with reduced risk of acute side effects in normal weight breast pts</td>
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<td>TGFβ1, SOD2, XRCC1, XRCC3, APEX</td>
<td>52*</td>
<td>TGFB1 codon 10 Pro allele &amp; TGFB1 position -509 T allele associated with increased risk of altered breast appearance</td>
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<td>De Ruyck 2005</td>
<td>XRCC1, XRCC3, OGG1</td>
<td>62</td>
<td>XRCC3 IVS5-14 associated with risk of late toxicity in cervix/endometrial pts, &gt;3 XRCC1 &amp; XRCC3 risk alleles → ↑ toxicity, XRCC1 codon 194 protective</td>
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<td>194Trp &amp; 399 Gln alleles together associated with toxicity in breast pts</td>
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<td>1853 Asn/Asn genotype associated with risk of acute &amp; late toxicity in breast pts</td>
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<td>RAD21</td>
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The importance of numbers: candidate genes vs whole genome

- ‘...SNP mining...the rush is on but how much gold will we find and how much pyrite?...’
- A candidate gene approach reduces complexity by targeting relevant genes and weighting the statistics in favour of finding important variations
- To achieve the statistical power for a genome wide association study (GWAS) up to 30,000 patients may be required

Genetic profiling is a rapidly changing field

- RAPPER - Radiogenomics: Assessment of Polymorphisms for Predicting the Effects of Radiotherapy
  - 2003 – conceived as a candidate gene study (60 genes with 5 SNPs/gene) using TaqMan technology
  - 2006 - able to analyse 120 candidate genes with 10 SNPs/gene using a SNPstream multiplex assay - a 4-fold increase for the same cost
  - 2008 - GWAS

Genome-wide association study (GWAS)

- Chromosomes are divided into haplotype blocks, separated by recombination hotspots
- Neighbouring SNPs are often strongly correlated in ‘linkage disequilibrium’
- Majority of common genetic variation can be evaluated using a few hundred thousand SNPs as tags for other variants

HapMap Project

- For ‘Weak Discouraged Men’ to ‘Bubble Over with Joyous Vitality’
- Rectal radium suppositories for ‘restoring sex power’
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RNA - expression microarrays

- cDNA microarrays first described in 1995 by a group in Stanford – Schena et al 1995 Science 270:467-70
- The ability to monitor the expression levels of thousands of genes / the whole genome in a single microarray
- Microarray methods require a combination of biological, mathematical and statistical expertise
- Different platforms – cDNA and oligonucleotide

Gene expression signature predicting outcome in patients with breast cancer

van de Vijver et al 2002

MammaPrint®

- 70-gene molecular profile
- Diagnostic test to assess the risk of breast cancer metastasis
- Based on Dutch gene signature
- FDA approved in 2007 for use in the U.S. for lymph node negative breast cancer patients under 61 years of age with tumours <5 cm
- Microarray based test that classifies tumours as low or high risk for recurrence

Prediction of radiotherapy response

Prediction of response to radiotherapy in cervical cancer. Results for the 300 genes that most correctly classified the samples into radiotherapy-resistant and -sensitive groups. Mean survival time after radiation treatment for the two groups:
- Radioresistant 22.2 mths
- Radiosensitive 66.5 mths

Wong et al 2003 Clin Cancer Res 9:5486-92

A 99-gene hypoxia associated signature

Winter, Buffa, Silva, West, Harris et al Cancer Research 2007; 67: .(7)
Relationship with prognosis in a published breast cancer series

Gene expression profiling
- Several microarray studies yielded gene sets whose expression profiles successfully predicted survival, but with almost no overlap between gene sets.
- Because of different technologies, patients and analysis methods?
- Many equally predictive lists can be produced from the same analysis.
- Reasons:
  - Many genes are correlated with survival
  - The differences between the correlations are small
  - The correlations fluctuate strongly when measured over different subsets of patients

Meta-signatures
- Analysis of four independent microarray studies involving different platforms (spotted cDNA, Affymetrix GeneChip, inkjet oligonucleotides) to derive an inter-study validated "meta-signature" associated with breast cancer prognosis.
- The individually identified gene sets predicted survival in each study, but had minimal overlap and performed poorly in pairwise cross-validation.
- The combined (n = 305 samples) 90-gene meta-signature strongly associated with survival in breast cancer patients.
- The meta-signature achieved comparable or better prognostic performance when compared with the individual signatures.
- Many of the "meta-signature" genes were involved in cell cycle control and signal transduction.
- Shen et al 2005 Genomics 5:94

Tissue microarrays – protein profiles

Molecular Marker Profiles Predict Locoregional Control of Head and Neck Squamous Cell Carcinoma in a Randomized Trial of Continuous Hyperfractionated Accelerated Radiotherapy

6
Molecular marker profiles

<table>
<thead>
<tr>
<th>Ki-67</th>
<th>TP53</th>
<th>CD31</th>
<th>Bcl-2</th>
<th>Cyclin D1</th>
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**CHART trial in HNSCC**

What else / next?
- Proteomics, whole genome comparative genomic hybridisation (CGH) arrays, whole genome methylation arrays
- miRNA signatures
- Exon arrays - most comprehensive coverage of the genome at RNA level analysing gene expression and alternative splicing. Possibility of detecting specific alternative splicing events that affect biological phenotypes.
- RNA-Seq, next generation sequencing, revolutionising transcriptomics, base level analysis, investigate expression of different gene alleles

Inter-patient variability: predictive assay, gene expression profiles

- The genomic revolution, ie sequencing of the human genome and developments in high throughput technology, has potential to enable us to predict patient response to radiotherapy
- There is a need to develop radiotherapy related phenotype specific meta-signatures
- Collaborative work is essential in order to carry out adequately powered studies
- We will need to explore how to incorporate the data within radiobiological models to improve radiotherapy dose prescriptions