Predictive Assays in Radiation Therapy

Radiation Biology
Lecture 4-23-2014

Outline
- Introduction
- Early predictive assays
- Recent trends in predictive assays
- Examples for specific tumors
- Summary

Introduction
- Absolute radioresistance does not exist: if a sufficiently high dose is delivered, all cells can be sterilized
- Radiation therapy objective is to optimize treatment for a higher probability of cure and minimal normal tissue damage
- Predictive assays are needed due to the potential role they could have in selecting individually tailored therapy course

Current clinical practice
- The radiation oncologist writes a prescription for
  - the total radiation dose in Gy
  - the dose per fraction
  - the number of fractions needed to deliver the total dose (and their temporal separation)
- These variables are mostly dictated by the primary site of disease, the histology and the stage of the cancer
- Geometrical factors are of utter importance: target should be fully covered, volume of exposed normal tissues minimized

Biological factors determining tumor response to radiotherapy
- There are three widely acknowledged radiobiological factors involved in determining tumor response to radiotherapy:
  - Cellular radiosensitivity
  - Tumor hypoxia
  - Cell proliferation rate
- Studies suggesting the potential of all three as prognostic factors for radiotherapy

Cellular Radiobiology Assays
- Not only tumors, but also normal tissues of individuals, differ in their intrinsic radiosensitivity
- Correlation between cellular radiosensitivity of skin fibroblasts and severe reaction to radiotherapy in an individual with the genetic disorder ataxia telangiectasia (A-T) was initially discovered in 1975
- Several independent studies shown a correlation between the in vitro radiosensitivity of skin fibroblasts and the severity of late complications
- A promising predictive assay?
Cellular Radiobiology Assays

- In the early 1990s, 1 study per year was published (black bars), all of them showing a significant relationship between in vitro radiosensitivity of fibroblasts and late effects of radiotherapy
- Two large confirmatory studies (white bars) published in 1998 and 2000 showed no significant predictive value of this assay for late effects

Early predictive assays

- Inherent radiosensitivity for normal tissue side effects is predictive in only small subset of tumors
- Proliferation rate (doubling time) looked promising in many small studies but turned out not to be a significant predictor of radiotherapy outcome in a larger multi-center analysis of 476 patients with head and neck squamous-cell carcinoma (HNSCC)
- Only the Eppendorf microelectrode measurement of partial oxygen tension has consistently shown to have prognostic value, recently confirmed in a joint analysis of outcome after radiotherapy in 397 patients with HNSCC from 7 centers

New era of predictive assays

- The cellular-based assays lacked the sensitivity and specificity
- New opportunity emerged through the Human Genome Project (2001 – 2003)
- Accompanying development of new high-throughput techniques provide extensive capabilities for the analysis of a large number of genes

DNA assays for normal tissue radiosensitivity

- It is now recognized that DNA mutations in a single or even a few genes are unlikely to be responsible for the patient-to-patient variability in sensitivity to radiation
- Single nucleotide polymorphisms (SNP) accounts for ~90% of the naturally occurring sequence variation within a population

DNA assays for tissue response

- Work carried out to date exploring genotyping to predict normal tissue and tumor response to radiotherapy has involved a candidate gene approach
  - uses a priori knowledge of SNP and gene functions
- Such approaches require smaller sample sizes and benefit from reduced complexity by targeting relevant genes
RNA microarrays

- Gene expression microarrays provide the ability to monitor, rapidly and simultaneously, the RNA expression levels of thousands of genes or the whole genome.
- Allows investigation of gene expression profiles associated with the radioresponse of tumors and normal tissues for the derivation of biomarkers to predict local control and toxicity after radiotherapy.

Proteomics and Tissue Microarrays

- The study of the function of all expressed proteins.
- The promise of proteomics lies in the identification of biomarkers that could favorably affect disease diagnosis, as well as our ability to assess the response to treatment and, thereby, the prognosis.
- Radioresistance-related proteins were identified in a proteomic study of pre-radiotherapy tumor biopsies from 17 patients with rectal cancer.

Controversial observations

- Example: the tumor suppressor gene p53
  - Mutations of p53 generally lead to deregulation of cell cycle by eliminating the G1 checkpoint, and impairment of DNA repair process.
  - Reported to be associated with increased cellular resistance to irradiation and tumor relapse after therapy.
  - The loss of p53 also shown to either increase or not change radiosensitivity of cells.
  - Current trend: the p53 protein is analyzed in normal and tumor cells for its functional quality.

Biomarker predictive assays

- Large studies are required with exploratory and validation cohorts of patients.
- Tissue banks are being established with the aim of collecting tissue from cancer patients linked with high-quality outcome data—obtained generally within the context of clinical trials.

Example: breast cancer

- At least 4 biologically distinct molecular subtypes of breast cancer were identified, which correlated to different clinical outcomes: luminal A (ER+, and/or PR+, HER2-), luminal B (ER+, and/or PR+, HER2+), HER2+(ER-, PR-, HER2+), and basal-like (ER-, PR-, HER2-).
- Drugs developed for ER+/PR+, and HER2+ patients make these subtypes easier to manage (tamoxifen, and trastuzumab or Herceptin).
### Example: breast cancer

- A study published by van’t Veer et al. (2002) described a 70-gene signature derived from a DNA microarray analysis of 78 young patients with BC that was associated with a short interval to distant metastases.
- The study was validated on a separate population of patients.
- Later, 76-gene classifier was developed in a similar group of patients by researchers from the Erasmus University in Rotterdam.
- Unfortunately, the 70- and the 76-gene signatures show relatively little overlap in terms of the genes selected: only 3 genes are common.

### Example: HNSCC

- The EGF-signaling pathway is of potential importance in radiation oncology because of its involvement in orchestrating the proliferative response of epithelial tumors to fractionated radiotherapy.
- EGFR (epidermal growth factor receptor) has been identified as oncogene.
- A large randomized phase III trial has shown that cetuximab, a monoclonal antibody against EGFR, significantly improves radiation therapy outcome in HNSCC.

### Current (2002) status of various predictive assays

<table>
<thead>
<tr>
<th>Assay</th>
<th>Brief description</th>
<th>Status under study</th>
<th>Technical difficulties</th>
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</thead>
<tbody>
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<td>Fluorescence attachment leukemia</td>
<td>Probes specific for leukemia (BC, MM)</td>
<td>Clinical</td>
<td>High</td>
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<tr>
<td>Lymphocyte drug sensitivity</td>
<td>Probes specific for lymphocyte drug sensitivity</td>
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<td>DNA microarray (MDA)</td>
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<tr>
<td>Immunohistochemistry</td>
<td>Probes specific for immunohistochemistry</td>
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<tr>
<td>Polymorphic p53</td>
<td>Probes specific for polymorphic p53</td>
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</tr>
<tr>
<td>Stains</td>
<td>Probes specific for stains</td>
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</table>

### Technical aspects and costs (2002)

<table>
<thead>
<tr>
<th>Method</th>
<th>Technical difficulties</th>
<th>Grade of difficulty (high/low)</th>
<th>Time to obtain result (d/week)</th>
<th>Reimbursement per sample (€)</th>
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</thead>
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<tr>
<td>Fluorescence attachment leukemia</td>
<td>High</td>
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<td>1</td>
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Summary

- Very few prognostic markers and virtually no predictive assays have been established in routine clinical radiation oncology
- New approaches concentrating on biological markers as opposed to cellular assays are promising due to possibility of acquiring large datasets with controlled parameters
- It is still not possible to draw conclusions regarding the most appropriate biologic endpoints to use within clinical trials or provide a template for future studies

References

- Predictive assays and their role in selection of radiation as the therapeutic modality, IAEA VIENNA, 2002
- S.N. Bentzen, From Cellular to High-Throughput Predictive Assays in Radiation Oncology: Challenges and Opportunities, Semin Radiat Oncol 18:75-88, 2008
- D.T. Miyamoto, J.R. Harris, Molecular Predictors of Local Tumor Control in Early-Stage Breast Cancer, Semin Radiat Oncol 21:35-42, 2011