Predictive Assays in Radiation Therapy
Immunotherapy in Cancer Treatment

Radiation Biology
Lecture 4-27-2020

Outline
- Introduction: Predictive assays in radiation therapy
- Examples for specific tumors
- Immunotherapy
- 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?
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.

Molecular (biomarker) tests have the potential to be more robust, comprehensive, and capable of better standardization between centers.
- These assays can be carried out in various clinical samples at the DNA (genome), RNA (transcriptome) or protein (proteome) level.

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) account for ~90% of the naturally occurring sequence variation within a population.
  - Up to 1% of the total of 3 billion bps.

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

DNA/RNA microarrays

- Done on “chips”
- Gene expression values from microarray experiments can be represented as heat maps to visualize the result of data analysis.

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

Biomarker predictive assays

- Large studies are required with exploratory and validation cohorts of patients, associated with the collection of high-quality physics, clinical and outcome data

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
Controversial observations

- Ki-67 protein is associated with proliferation, cell does not progress through division without this protein generated.
- It is a prognostic parameter, related to disease-free and overall survival, especially for breast cancer patients.
- It is not a predictive parameter, so far no correlation with efficacy of a specific chemo agent, etc. has been established.

Example: breast cancer

<table>
<thead>
<tr>
<th>Year</th>
<th>Median Follow-up</th>
<th>Breast</th>
<th>Luminal A</th>
<th>Luminal B</th>
<th>HER2</th>
<th>HER2+ Breast</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-year LR</td>
<td>760</td>
<td>76</td>
<td>6.6</td>
<td>5.5</td>
<td>6.4</td>
<td>5.3</td>
</tr>
<tr>
<td>3-year LR</td>
<td>458</td>
<td>94</td>
<td>1.0</td>
<td>4.3</td>
<td>7.7</td>
<td>0.6</td>
</tr>
<tr>
<td>5-year LR</td>
<td>1,401</td>
<td>194</td>
<td>8</td>
<td>19</td>
<td>21</td>
<td>14</td>
</tr>
<tr>
<td>10-year LR</td>
<td>3</td>
<td>6</td>
<td>14</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kaplan-Meier plot of disease-free survival in years based on Ki-67 categories</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

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+
- Basal-like (ER-, PR-, HER2-), also called "triple negative"

Example: prostate cancer

- Novel gene-based tests have been developed to improve the prediction accuracy at various phases within the prostate cancer (PCa) disease course.
- Urine-based assays (expression levels of PCA3 and TMPRSS2:ERG) aim to refine the selection for both initial and repeat prostate biopsy.
- Tissue-based gene expression tests: to predict the occurrence of subsequent PCa events, including adverse characteristics, biochemical recurrence, metastatic progression, and mortality.

Immunological markers that predict radiation toxicity

- Radiotoxicities can be generally classified into two major groups, ‘early’, and ‘late’ (months to years following treatment).
- Late adverse effects are more critical:
  - They are persistent and often progressive
  - May have severe and debilitating effects (e.g., fibrosis, necrosis, atrophy, vascular changes, telangiectasia, secondary malignancies)
  - Can be fatal in some instances

Immunological markers that predict radiation toxicity

- Therapeutic doses of radiation lead to large amounts of cellular damage; the immune response plays a major role in dealing with it.
- The resident immune cells produce pro-inflammatory cytokines and growth factors, eventually leading to chronic inflammation, which may induce the genomic instability which in turn perpetuates the inflammation.
Immunological markers that predict radiation toxicity

- Modulating immune cells during the radiation-induced inflammatory response may provide benefits to avoid a severe fibrosis outcome.
- Several studies for different cancer types implicate immunological markers for radiation sensitivity such as transforming growth factor TGFβ and associated genes.

Current (2002) status of various predictive assays

<table>
<thead>
<tr>
<th>Assay</th>
<th>Brief description</th>
<th>Status under clinical evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor size-related factor (TFR)</td>
<td>Progression of tumor size-related factors measured with TFR methods.</td>
<td>Clinical</td>
</tr>
<tr>
<td>Estrogen receptor (ER)</td>
<td>Estimation of estrogen receptor level in breast cancer tissue.</td>
<td>Clinical</td>
</tr>
<tr>
<td>Progesterone receptor (PR)</td>
<td>Estimation of progesterone receptor level in breast cancer tissue.</td>
<td>Clinical</td>
</tr>
<tr>
<td>HER2</td>
<td>Estimation of HER2 protein expression in breast cancer tissue.</td>
<td>Clinical</td>
</tr>
<tr>
<td>Nuclear factor kappa B (NF-kB)</td>
<td>Estimation of nuclear factor kappa B activity in breast cancer tissue.</td>
<td>Clinical</td>
</tr>
<tr>
<td>Hypoxia-inducible factor alpha (HIF-α)</td>
<td>Estimation of HIF-α protein expression in breast cancer tissue.</td>
<td>Clinical</td>
</tr>
</tbody>
</table>

Technical aspects and costs

<table>
<thead>
<tr>
<th>Method</th>
<th>Technical details</th>
<th>Grade of evidence (high/low)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue-embedded mass spectrometry</td>
<td>Mass spectrometry analysis of tissue samples</td>
<td>High</td>
</tr>
<tr>
<td>Polymerase chain reaction (PCR)</td>
<td>Amplification of specific DNA sequences</td>
<td>Low</td>
</tr>
<tr>
<td>Microarray analysis</td>
<td>Analysis of gene expression profiles</td>
<td>Low</td>
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</tbody>
</table>

Current (2019) breast cancer genomic tests

- The Breast Cancer Index test analyzes the activity of 7 genes that influence how likely the cancer is to recur in 5 to 10 years after diagnosis, and how likely a woman is to benefit from 5 additional years of hormonal therapy.
- The EndoPredict test is used to predict the risk of distant recurrence of early-stage, hormone-receptor-positive, HER2-negative breast cancer that is either node-negative or has up to three positive lymph nodes.
- The Prosigna Breast Cancer Prognostic Gene Signature Assay (formerly the PAM50 test) predicts the risk of recurrence of DCIS and/or the risk of a new invasive cancer developing in the same breast, and benefits from radiation after surgery.
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Immunotherapy in treatment of cancer

- Test for levels of PD-L1 (programmed death ligand 1) in tumor, if >50% of cells, use of anti-PD-L1 agents should be successful.

- Several immune checkpoint inhibitors have been approved by the FDA.
- The first such drug to receive approval, ipilimumab (Yervoy), for the treatment of advanced melanoma.
- Other drugs, targeting different checkpoint inhibitors are: nivolumab (Opdivo) and pembrolizumab (Keytruda); approved for treatments of advanced melanoma or advanced lung cancer.

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Immunotherapy: new role of radiation therapy

- Standard approach: radiotherapy effects on survival of cancer patients are generally interpreted as the consequence of improved local control of the tumor, directly decreasing systemic spread.
- Experimental data from multiple cancer models have provided sufficient evidence to propose a paradigm shift: some of the effects of ionizing radiation are recognized as contributing to systemic antitumor immunity.

Immunotherapy: new role of radiation therapy

- Example: two metaanalyses of prospective, randomized trials in breast cancer demonstrated a direct contribution of adjuvant radiotherapy to patients’ long-term survival; the effect was independent of stage and extent of surgery.
- Possible explanation: Radiotherapy engages both the innate and adaptive arms of the immune system, with the potential to convert the irradiated cancer into an in situ vaccine that elicits tumor-specific T cells.

Immunotherapy: abscopal effect with RT

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Immunotherapy: abscopal effect with RT

- Why does it work so rarely? Progression of cancer is possible through escape from immune system
- Balance between pro-immunogenic and immunosuppressive effects
- Current status: pre-clinical

Immunotherapy in treatment of cancer

- Cost is prohibitive for many patients:
  - 12 new oncology treatments approved in 2012, 11 were priced above $100,000 for one year of treatment
  - Opdivo, approved for both melanoma and lung cancer, is priced at $12,500 a month, or about $150,000 for a year of treatment; Keytruda, approved for the treatment of metastatic melanoma, costs about the same
  - Provenge (sipuleucel-T), a series of 3 immunotherapy vaccines approved in 2010; improves median overall survival of men with advanced prostate cancer by 4.1 months, is priced at $93,000 per patient
  - Patients take the drug until disease progression or unacceptable toxicity

Summary

- Despite a substantial research effort over 25 years, 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
- Immunotherapy is a fast-growing and promising field; so far works only for limited number of patients

References

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- http://www.cancer.gov/research/areas/treatment/immunotherapy
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Beware of the bystander effect!