Disclosures

In addition to my faculty appointment at UNC, I also teach radiation and cancer biology at other institutions on a freelance, consultancy basis.
Learning Objectives

• Grasp – in a qualitative sense – the molecular and cellular biology of cancer, and how this manifests in the behavior of malignant tumors as a whole.

• Describe the biological underpinnings of molecular imaging, and how it can facilitate the detection, characterization and ultimately, the eradication of, tumors containing therapy-resistant cells.

• Better understand why chemotherapy is often given concurrently with radiotherapy as a means of producing tumor radiosensitization.

Cancer Biology Primer

Just what is cancer, anyway?

Answer: It depends on who you ask…

An Oncologist’s Definition: “A spectrum of many different diseases, usually of multiple etiologies, that if left untreated, almost always results in the death of the patient either directly or indirectly.”
Cancer Biology Primer

A Pathologist’s Definition: “A relatively autonomous growth of tissue that negatively affects the structure or function of the tissue of origin, and that is also capable of invasive and/or metastatic behavior.”

Cancer Biology Primer

A Cell Biologist’s Definition: “A disease of the cell in which the normal mechanisms of cell proliferation, death, differentiation, motility and/or communication are dysregulated secondary to deleterious changes in the cell’s DNA.”
Cancer Biology Primer: The Hallmarks of Cancer

1. GROWTH EVEN IN THE ABSENCE OF NORMAL “GO” SIGNALS
Most normal cells wait for an external message before dividing. Cancer cells (image) often counterfeit their own pro-growth messages.

2. GROWTH DESPITE “STOP” COMMANDS ISSUED BY NEIGHBORING CELLS
As the tumor (yellow) expands, it squeezes adjacent tissue, which sends out chemical messages that would normally bring cell division to a halt. Malignant cells ignore the commands.

Cancer Biology Primer: The Hallmarks of Cancer

3. EVASION OF BUILT-IN AUTODESTRUCT MECHANISMS
In healthy cells, genetic damage above a critical level usually activates a suicide program. Cancerous cells (magenta) bypass this mechanism, although agents of the immune system (orange) can sometimes successfully order the cancer cells to self-destruct.

4. ABILITY TO STIMULATE BLOOD VESSEL CONSTRUCTION
Tumors need oxygen and nutrients to survive. They obtain them by co-opting nearby blood vessels to form new branches (brown streaks) that run throughout the growing mass.
Cancer Biology Primer: The Hallmarks of Cancer

5. EFFECTIVE IMMORTALITY
Healthy cells can divide no more than 70 times. Malignant cells need more than that to make tumors. So they work around systems—such as the telomeres (yellow) at the end of chromosomes (blue)—that enforce the reproductive limit.

6. POWER TO INVADE OTHER TISSUES AND SPREAD TO OTHER ORGANS
Cancers usually become life-threatening only after they somehow disable the cellular circuitry that confines them to a specific part of the particular organ in which they arose. New growths (orange and yellow) appear and eventually interfere with vital systems.

But what is driving normal cells to start exhibiting these aberrant “behaviors” that together culminate in a malignant tumor?

Answer: Gradually-accumulating damage to DNA’s structure, function and/or regulation.
Cancer Biology Primer

Why? How?

Answer: Because another, overriding hallmark of cancer is that the DNA of malignant cells exhibits “genomic instability”, a state of hyper-mutability that leads to the accumulation of more and more errors.

Cancer Biology Primer

What key genes get mutated?

Oncogenes may become activated, genes whose products tend to:

- (over-) promote cell proliferation and motility;
- lead to resistance to various types of cancer therapy; and
- confer reproductive immortality
Cancer Biology Primer

Or:

**Tumor suppressor genes may be inactivated or lost**, genes whose products tend to:

- reign in excessive cell growth and proliferation;
- maintain the integrity of DNA and establish reproductive time limits for cells;
- ensure that irreparably damaged cells commit suicide rather than propagate undesirable traits; and
- help coordinate complex cellular responses to rapidly changing environmental conditions

Cancer Biology Primer

Cells communicate with, and respond to, their microenvironment using a process called **signal transduction**.

It shouldn’t be so surprising that **many oncogene and tumor suppressor gene proteins take part in signaling pathways**.
Cancer Biology Primer

Cell signaling (cont.)

Signaling is “...an enzymatic cascade that converts a mechanical or biochemical stimulus to the outside surface of the cell into a specific cellular response instigated in the nucleus.” (paraphrased from Wikipedia)

Sounds simple enough, right?

Cancer Biology Primer

Not really, no.

Signaling can be exceedingly complex, with many proteins involved in multiple, interrelated pathways.

Upside: rapid, flexible response to "stress"

Downside: one protein goes bad, and the whole process can unravel…
…which could be especially problematic if the protein that went bad was a key controller of multiple pathways, called a “caretaker”.

These constantly evolving changes in the genomes of tumor cells can confer intrinsic resistance to cancer therapy.

Once formed though, tumors as a whole can also develop forms of extrinsic resistance.
Cancer Biology Primer
Extrinsic or “microenvironmental” heterogeneity of tumors

Why?

Answer: **Because tumor vasculature is abnormal.**

- Abnormal Structurally
- Abnormal Functionally
- Abnormal Physiologically
- Abnormal Angiogenesis
Cancer Biology Primer

The combination of abnormal vasculature and the tendency of tumors to outgrown their own blood supply can lead to extrinsic resistance to cancer therapy in the form of **tumor hypoxia**.

Hypoxic cells are:
- radiation resistant
- often chemotherapy resistant
- more aggressive overall

Cancer Biology: Clinical Implications

- By the time even the smallest tumor is diagnosed, it contains as many as a billion cells, most of which are already quite diverse with respect to their future behavior and response to treatment.

- Therapy resistance can be either an intrinsic cellular property or result from features of the tumor’s microenvironment. Especially troublesome cell types include:
  - *inherently resistant cells*
  - *rapidly proliferating cells*
  - *hypoxic cells*

- Radiation and chemotherapy both attempt to target one or more of these troublemakers.
Cancer Biology: Clinical Implications

• It would be really, really helpful to know in advance which type(s), and how many, resistant tumor cells are present in a particular patient’s tumor prior to treatment.

• It would also be useful to monitor such cell populations during and after treatment, and determine whether doing so has any predictive value.

• The aberrant molecular features of such cells can be used against them: either as a means of identifying their presence; or as targets for the development of new treatments.

Session Speakers

Dr. Humm: “PET Imaging of Tumor Hypoxia”

• The Target: Radiation and chemotherapy-resistant hypoxic tumor cells.

• The Problem: Can their presence in human tumors be detected and quantified…preferably, in a non-invasive way?

• The Approach: Compare and contrast different hypoxic cell biomarkers and detection methods, with emphasis on PET probes that can be used for non-invasive imaging.

• Long Term Goal: Bio-dose “painting” for treatment planning
Imaging of Tumor Hypoxia

Several properties of hypoxic cells make them suitable targets for imaging:

- **Environmental characteristics**: oxygen concentration, pH, relative vascularity, presence of proliferating cells, etc., in micro-regions of tumors
- **Biochemical characteristics**: cells in a low-oxygen environment rely on different biochemical pathways to generate energy and break down complex molecules than well-oxygenated cells do
- **Genetic characteristics**: different genes and proteins are active in hypoxic cells than aerated ones

Imaging of Tumor Hypoxia

Markers of an hypoxic tumor microenvironment?

Low oxygen tension → Oxygen electrode
(“Eppendorf” or “OxyLite”)

**Pros:**
- Direct measure of oxygen tension in micro regions of tissue
- Correlates with clinical outcomes in some cases

**Cons:**
- The very definition of “invasive procedure”
- Not really imaging *per se*
- Lots of technical nuances
Imaging of Tumor Hypoxia

As measured using oxygen electrodes, more hypoxic tumors have worse outcomes after radiation therapy than less hypoxic tumors.

Imaging of Tumor Hypoxia

Markers of hypoxic biochemistry and metabolism?

Nitroimidazoles → Selectively broken down, only under hypoxic conditions, to form products that bind to other cellular molecules

These breakdown products can be tagged for imaging purposes...

- Colorimetric dye tag – for histology/pathology specimens
- Radioactive tag – for autoradiography, PET, SPECT
- Fluorescent (antibody) tag – for immunohistochemistry
Imaging of Tumor Hypoxia

Popular nitroimidazole hypoxia markers include:

- **Hypoxyprobe-1™** (pimonidazole hydrochloride) – its metabolic breakdown products are detected in hypoxic cells using an antibody

![Hypoxia detected in a rodent tumor cell spheroid model, using pimo tagged with a red stain](image1.png)

Imaging of Tumor Hypoxia

Popular nitroimidazole hypoxia markers include:

- **EF-5** – its metabolic breakdown products can be detected in hypoxic cells using an antibody, a radioactive tracer (for autoradiography or PET) or by MRS/MRI detection of fluorine atoms

![Hypoxia staining with EF-5 (red) and a marker for blood vessels (green) in a rodent breast tumor](image2.png)
Imaging of Tumor Hypoxia

Other nitroimidazole or related compounds for the detection of cellular hypoxia:

- **CCI-103F** – a hexafluorinated (¹⁹F) analog of misonidazole for MRI/MRS

- **¹⁸F-Miso** – a (radioactive) fluorinated analog of misonidazole for PET and SPECT

- **¹²⁴I-AZA/¹⁸F-AZA** – azomycin arabinoside analogs of misonidazole for PET

**Imaging of Tumor Hypoxia**

Pros and Cons: Nitroimidazole-derived hypoxia markers

**Pros**
- Detect hypoxia at the cellular level
- Semi-quantitative
- Require metabolic processing (i.e., dead cells won’t stain)
- Can look at interrelationships with other tumor markers in a “geographic” sense

**Cons**
- Exogenous chemicals (drug must be administered to patients in advance)
- Most experience to date involves biopsy-based methods rather than non-invasive imaging
- Reproductively dead cells might still stain
Imaging of Tumor Hypoxia

Markers of hypoxia-related genes and proteins?

Many different types of genes are activated and protein production increased in response to cellular stress caused by hypoxic conditions. These include genes and proteins related to:

- Glucose metabolism (examples: GLUT-1, CA-9)
- Angiogenesis (example: VEGF)
- Cellular motility and tissue remodeling (examples: OPN, LOX)
- Evasion of cell death (example: Bcl-2)
- Transcription factors that turn on other genes (example: HIF-1α)

Imaging of Tumor Hypoxia

The presence of these proteins in cells can be detected and used as a surrogate marker for tumor hypoxia.
Imaging of Tumor Hypoxia

Pros and Cons: Endogenous hypoxia markers

**Pros**
- Detect hypoxia at the cellular level
- Semi-quantitative
- Endogenous proteins
  - no drug to give to patients
  - can be used on archival specimens
- Can look at interrelationships with other tumor markers in a “geographic” sense

**Cons**
- Reproductively dead cells might still stain
- Some of these genes and proteins are also expressed in response to cell stressors other than hypoxia
- Different staining patterns and intensities for different markers

But how does the cell “sense” that it’s hypoxic, and that it needs to activate certain genes and make new proteins in order to cope?

**Answer:** Cells have a built-in oxygen “barometer” in the form of a protein called HIF-1α.

This protein is only stable under hypoxic conditions, and serves as a transcription factor to turn oxygen-regulated genes on and off as needed.
Imaging of Tumor Hypoxia

The activation of genes by HIF-1α can itself be imaged, using a reporter gene assay.

Reporter genes are constructed in the lab and then introduced into cells. A reporter consists of the regulatory or promotor portion of one gene linked to the coding sequence of a different gene that produces a protein that can be imaged.

Imaging of Tumor Hypoxia

• Illustrating the use of a reporter gene (GFP) to identify the early development of hypoxia in a newly-implanted rodent tumor.

• Prior to implantation, tumor cells were engineered to contain a reporter consisting of the gene for GFP, controlled by the hypoxia responsive element promotor region, which is borrowed from genes only activated under hypoxic conditions.
Session Speakers

Dr. Dicker: “Combinations of Chemotherapy and Radiotherapy”

- The Target: Intrinsically resistant and/or rapidly proliferating tumor cells
- The Problem: Can their negative effects on tumor control be neutralized through rationally-designed combinations of radiation and chemotherapy?
- The Other Problem: How can anything be “rationally-designed” when we barely know what we’re talking about?
- Long Term Goal: Improve the therapeutic ratio

Chemoradiotherapy

Why combine radiotherapy with chemotherapy?

Answer: To achieve better tumor control (both locally and at distant sites) than can be achieved by either radiation or chemotherapy alone, and preferably, while staying within the limits of normal tissue toxicity.
Chemoradiotherapy

How?

Answer: By following a few guiding principles…

1. **Spatial Cooperation** – when the radiation targets one part of the tumor, and the chemotherapy another

![Spatial Cooperation Diagram](image)

2. **Toxicity Independence** – when the radiation and chemotherapy have different dose-limiting normal tissue toxicities, such that both can be given full dose without further exacerbating damage to either tissue

![Toxicity Independence Graph](image)

3. **Radiation Protection** – when the chemotherapy drug is not particularly toxic in and of itself, but rather increases the normal tissue’s (not tumor’s) tolerance to radiation (example: amifostine)
Chemoradiotherapy

Radiation Sensitization – regardless of whether the chemotherapy agent is toxic in and of itself, it also has the property of increasing a tumor’s radiation sensitivity (and probably, that of irradiated normal tissue too)

- When radiosensitization is the main goal, the drug is usually given concurrently with the radiation (e.g., 5-fluorouracil, cisplatin, gemcitabine)

Chemoradiotherapy

What is it about a chemotherapy drug that would make it “synergize” with radiation?

Short Answer: In many cases, we don’t really know.

Long Answer: There are plenty of possibilities as to why it should, yet definitive mechanisms of action can be hard to come by.
Chemoradiotherapy

Possibilities:

- Damage caused by radiation and drug exacerbate each other
- Drug inhibits the repair of radiation-induced DNA damage
- Drug triggers cellular suicide (apoptosis) whereas radiation usually doesn’t
- Drug and radiation are preferentially toxic to cells in different phases of their cell cycle, such that the toxicities complement each other

DNA synthesis

\[ G_2 \]
Growth, energy generation and synthesis of components needed for mitosis

\[ G_1 \]
Growth, energy generation and synthesis of components needed for DNA synthesis

\[ S \]
DNA synthesis

Etoposide, bleomycin, actinomycin D, radiation

Vincristine, taxol

5-fluorouracil, methotrexate, gemcitabine

Even distribution of genetic material and cell division
Chemoradiotherapy

Possibilities (cont.):

- Drug helps counteract tumor cell proliferation that occurs between radiation doses
- Tumor cell killing by either modality decreases O$_2$ consumption/shrinks tumor/increases blood flow, so that both oxygenation and drug access improve

Reperfusion of a human laryngeal carcinoma grown in nude mice after a large single radiation dose.

Pre-irradiation 7 hours after 10 Gy

Hypoxia = green
Blood vessels = red/pink
Perfusion pattern = blue


Chemoradiotherapy

Barriers that impede the *rational* design of chemoradiotherapy protocols:

- Unlike radiation, the concept of drug “dose” is vague

Distribution of doxorubicin (blue) relative to location of the tumor vasculature (pink) in a human MCF-7 breast tumor grown in nude mice. Note that the drug does not fully penetrate throughout the tumor mass.
Chemoradiotherapy

Barriers (cont.):

- The mechanisms of action for many drugs remain poorly understood – both in terms of toxicity and radiosensitization.

<table>
<thead>
<tr>
<th>Barriers (cont.):</th>
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<tbody>
<tr>
<td>- There is a much greater variability in tumor cell response to drugs than to radiation in terms of toxicity; is this also true for radiosensitization?</td>
</tr>
</tbody>
</table>

Response of different cell types to one drug (taxol).

Response of one cell type (human gastric cancer) to different drugs.
Barriers (cont.):

- We know that the sequencing/timing of the drugs and radiation is important, but we don’t necessarily know why, or how best to optimize this.

Because chemotherapy is typically not specific for tumor versus normal cells, we know that normal tissue reactions can also be enhanced by combined chemoradiotherapy...early effects usually, but late effects?

Summary of the preclinical data regarding the toxicity of concomitant chemoradiation:

<table>
<thead>
<tr>
<th>Agents</th>
<th>Early effects</th>
<th>Late effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antimetabolites:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-Fluorouracil</td>
<td>(GI, skin)</td>
<td>?</td>
</tr>
<tr>
<td>Mitomycin C</td>
<td>(GI)</td>
<td>?</td>
</tr>
<tr>
<td>Hydroxyurea</td>
<td>(GI)</td>
<td>?</td>
</tr>
<tr>
<td>Gemcitabine</td>
<td>(GI)</td>
<td>?</td>
</tr>
<tr>
<td>Fluorodeoxyuridine</td>
<td>(GI)</td>
<td>? (lung)</td>
</tr>
<tr>
<td>Platin derivatives</td>
<td>(GI, BM)</td>
<td>?</td>
</tr>
<tr>
<td>Topotecan</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Taxol</td>
<td>(GI)</td>
<td>?</td>
</tr>
<tr>
<td>Others</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Docetaxel</td>
<td>(GI, skin)</td>
<td>? (liver, lung)</td>
</tr>
<tr>
<td>Mitomycin C</td>
<td>(GI, BM)</td>
<td>? (lung)</td>
</tr>
<tr>
<td>Imitaxol</td>
<td>(GI, skin)</td>
<td>? (lung)</td>
</tr>
<tr>
<td>Mitomycin C-6D</td>
<td>(GI, BM, skin)</td>
<td>? (lung)</td>
</tr>
<tr>
<td>Alkylating agents</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cisplatin</td>
<td>(GI)</td>
<td>? (liver)</td>
</tr>
<tr>
<td>BORU</td>
<td>(GI)</td>
<td>? (lung)</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>(GI, skin)</td>
<td>? (lung, bladder, CNS)</td>
</tr>
</tbody>
</table>

BORU, 5-bromo-5-nitro-2'-deoxyuridine; BM, bone marrow; CNS, central nervous system; GI, gastrointestinal.
- Not demonstrated; +, demonstrated; =, conflicting data; ?, unknown.
Summary/Conclusions

Many of the very properties – molecular, cellular or physiological – that make cancer cancer can also be co-opted for imaging purposes.

Once imaging is possible, the door is open for further studies ranging from:

• basic cancer cell and tumor biology;
• to the development of new cancer drugs;
• to predictive assays of treatment progress and outcome; and
• to new and rationally-designed clinical trials