October 31, 2009

“When Models Go Awry: DAMPs and Autophagy in Pancreatic Cancer”

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Model Checking as a verification technique for finite state concurrent systems. His research group pioneered the use of Model Checking for hardware verification. Symbolic Model Checking using BDDs was also developed by his group. In addition, his research group developed the first parallel resolution theorem prover (Parthenon) and the first theorem prover to be based on a symbolic computation system (Analytica).
Now – A Pausch Bridge
Deaths from cancer, adjusted for the size and age of the population, have changed little since the 1950s, while death rates from heart disease and stroke have dropped significantly.
Cancer is a “Wicked Problem”

Cancer is a "Wicked Problem"

- One that is difficult or impossible to solve because of incomplete, contradictory, and changing requirements that are often difficult to recognize.
- Because of complex interdependencies, the effort to solve one aspect of a wicked problem may reveal or create other problems.
Still Deadly

The death rate from cancer fell only 5 percent from 1950 to 2005, in contrast to the large declines seen in other major killers like heart disease and stroke.

Source: National Center for Health Statistics
Survival after Resection of Adenocarcinoma of the Pancreas

Actuarial 5yr = 20%
Actual 5yr = 17%

Cancer and Inflammation

Rudolf Virchow suggested that the origin of cancer was in sites of chronic inflammation since 1863.

<table>
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<tr>
<th>Chronic inflammation</th>
<th>Associated cancer</th>
<th>Aetiological agent</th>
<th>Percent predisposed that progress to cancer</th>
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<tr>
<td>Bronchitie</td>
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</tr>
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<td>Cervicitis</td>
<td>Cervical cancer</td>
<td>Human papillomavirus</td>
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<td>Non-melanoma skin cancer</td>
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</tr>
<tr>
<td>Inflammatory bowel disease</td>
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<td>Gut pathogen, altered gut permeability</td>
<td>1*</td>
</tr>
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<td>Pancreatitis</td>
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<td>Tobacco, genetic factors</td>
<td>≤10%†</td>
</tr>
<tr>
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<td>Gastric acid, alcohol, tobacco</td>
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*Per year. †In susceptible populations. ‡At cholecystectomy.
# Chronic Inflammatory Conditions Associated with Cancer


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Multianalyte profiling of serum cytokines for detection of pancreatic cancer


Area under the ROC: 0.986062

Chronic Inflammation and Cancer

Mode of Cell Death is Important for Immune Cell Recruitment and Activation

- Damaged or Dying Cells
- Secreted From Stressed Cells
- Protease
  - PMN
  - Degradation of Tissue Matrix

Pattern Recognition Receptor

- i.e. HMGB1
- i.e. RAGE

Angiogenesis

Nuclear translocation NF-kB, pERK1/2

Initiation of cell repair

Tumorigenesis, metastasis

Endothelial cell activation, smooth muscle cell migration, mesoangioblast migration/proliferation

Pathogen-associated Molecular Patterns (PAMPs): Molecules expressed or released by invading microorganisms that are structurally unique to the pathogen.

Ruslan Medzhitov, 2000

Damage-associated Molecular Patterns (DAMPs): Molecules expressed or released that are normally unavailable to the immune system but are released and recognized by immune cells following tissue injury [Danger].

Polly Matzinger, 1995
DAMPs - Chronic Tumor Lysis Syndrome

Cell Constituents:

- HMGB1 – Cytochrome C
- Heat shock proteins
- Uric Acid, ATP, Adenosine; CpG DNA
- s100 proteins
- Hepatoma derived growth factor
- LDH
- DNA

Secreted molecules:

- Fibrinogen domain A
- Surfactant protein A

Matrix elements:

- Heparan sulfate
- Soluble hyluranan
- Fibronectin

Pancreatic Tumor Progression

**Signal**
- Growth Factor
- Stress

**Mode of Cell Death**
- Autophagy
- Apoptosis

**Metabolism**
- Oxidative phos.
- Glycolysis
- Oxidized
- Reduced

**Microenvironment**
- Normal
- PANIN 1-3
- Pancreatic Ca

**Host Immune Response**
- Tolerance
- Inflammation
- Suppression
DAMPs in Pancreatic Cancer

- Death is a rather constant concomitant of Pancreatic Cancer
- Current clinical protocols UPCI [Zeh]
- Death Pathways [Apoptosis, Autophagy, Necrosis]
- DAMPs
- HMGB1
- Novel Therapeutic Strategies Targeting Autophagy
Resources in Pancreatic Cancer

- Clinical Program with 450 new cases/year of Pancreatic Cancer; Robotic Program [WTAE]
- Current clinical protocols UPCI [Zeh, Moser, Bahary, Lembersky, Lotze, Whitcomb, Normolle]
- Subcutaneous and Orthotopic Pancreatic Tumors
- PDX-cre Mutant Kras Spontaneous Tumor Model
- Imaging and Flow Cytometers [Buchser]
- ForteBio Interferometer
- Seahorse Measures of OXPHOS and Glycolysis
- Targeting Autophagy
- miRNA, proteomics and transcriptomics
Extent of Disease at Diagnosis

Shaib et al, Aliment Pharmacol Ther 24, 87-94, 2006
Survival after Resection of Adenocarcinoma of the Pancreas

Actuarial 5yr = 20%
Actual 5yr = 17%

**Neoadjuvant Phase II Trial: UPCI 06-035**

- **Investigator-initiated (Genentech)**
  - Largest trial of its kind in the world (n=60)
  - *Nationwide* prospects (25 centers interested)

- **Dual primary (objective) endpoints:**
  - Margin negative resection rate
  - Complete pathologic response rate

- **Treated tissue is the key to future trial development**
  - Pathology, cancer genetics, gene expression
  - 35/60 enrolled; opened 2/08
The Pancreatic Cancer Research Team (PCRT) is a collaboration of international researchers with the mission to organize and accelerate the clinical development of new agents for the treatment of patients with pancreatic cancer. PCRT offers a central resource for patients seeking the most up-to-date clinical trials. TGEN – Dan Von Hoff and Ramesh Ramanathan. New U01 Funding with Tim Wang at Columbia and Jamie Lee at Scottsdale Mayo Clinic [$10M].
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<th>Cancer Type</th>
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<th>p-Value</th>
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<tr>
<td>Mesothelioma</td>
<td>Edwards, 2003</td>
<td>0.008</td>
</tr>
<tr>
<td>Renal-clear cell carcinoma</td>
<td>Cheville, 2003; Tollefson, 2007</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Colon carcinoma</td>
<td>Hunter, 1983</td>
<td></td>
</tr>
<tr>
<td>NSCLC</td>
<td>Swinson, 2003</td>
<td>0.0016</td>
</tr>
<tr>
<td>Breast</td>
<td>Gilchrist, 2003; Kato, 2002</td>
<td>0.0068</td>
</tr>
<tr>
<td>Mucosal melanoma</td>
<td>Prasod, 2002</td>
<td>0.007</td>
</tr>
<tr>
<td>Melanoma</td>
<td>Balch, 2001</td>
<td></td>
</tr>
<tr>
<td>Sarcoma</td>
<td>Miyajima, 2002; Gustafson, 2003</td>
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Very low -- The absence of autophagy increases cell death during metabolic stress and on treatment with cytotoxic chemotherapeutic agents.

Intermediate -- Physiological levels of autophagy are essential for normal cellular homeostasis.

Very high -- excessive levels of autophagy promote cell death.

Nature 446, 745-747 (12 April 2007)
Beth Levine
Distinct autophagic phases:

I. Autophagosome formation — Sequestration
II. Degradation
III. Utilization — Provision of amino acids
Cross-Regulation Between Autophagy and Apoptosis

HMGB1 Knockout Mice die soon after birth as a result of lethal hypoglycemia

Day 25

HMGB1 -/-

Nature Genetics 22, 276 - 280 (1999)

Marco E. Bianchi Lab
bianchi.marco@hsr.it
Measuring Oxygen Consumption Rate (OCR) and Extracellular Acidification Rate (ECAR)

The bio-cartridge is raised, bringing the system back to baseline micro-chamber is formed

The rate is calculated from the slope

Ben Van Houten
Knockout of HMGB1 Impairs Mitochondrial Function

A: Oligomycin
B: FCCP
C: 2DG
D: Rotenone

A: Protein expression of HMGB1 and Actin

B: ATP content and MitSox content graphs for different treatments:
- HMGB1 WT
- HMGB1 KO
- KO+Vector
- KO+Plasmid

C: OCR (pMoles/min) and ECAR (mM/mH/min) graphs over time:
- HMGB1 WT
- HMGB1 KO
- KO+Vector
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Tumor Progression

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1. H M G B 1
2. H M G B 1
3. 4