Cancer Is A Disorder of Cell Death: Biologic Impact of Targeted Therapies

Michael T. Lotze, MD
Vice Chair and Chief, Translational Research
Departments of Surgery and Bioengineering
University of Pittsburgh Cancer Institute
Effect of tumour cells killed by x-rays upon the growth of admixed viable cells.

Tumor Incidence  Survival Curves

Stimulation Exerted by Dead Cells

• Specific stimulation by homologous cell products
• A ‘feeder effect’ in which the dead cells release essential nutrients
• Stimulation through provoking an inflammatory response and/or vascularization from the side of the host
TLRs, NLRs, and RLRs

Review

TIR, CARD and PYRIN: three domains for an antimicrobial triad

Cancer
Death Used to be Simpler
Apoptosis [I], Autophagy [II] and Necrosis [III]

Extrinsic
- TNF, LTα
- TRAIL, FasL

Intrinsic
- p53/PUMA
- RT, ChemoRx
- Mitochondrial
- Toxins, UV

Cytolytic –T/NK
- Perforin
- Granzymes A, B, K, M

Necrosis [III]

p53 Sequestration
- BCL2, BCLxL↑
- IAP, XIAP, Survivin

Autophagy [II]
- Beclin 1, LC3

Zeh H. J., 3rd and Lotze M. T.
Addicted to death: invasive cancer and the immune response to unscheduled cell death
### Classical Model
- **Type 1:** Apoptosis
- **Type 2:** Autophagy
- **Type 3:** Necrosis

G. Mollino, P. Nicotera et al., *Cell Death and Differentiation*, 12 (2005)

### Current Model
- **11 forms** of cell death

#### Diagram

```
<table>
<thead>
<tr>
<th></th>
<th>NECROSIS</th>
<th>APOPTOSIS</th>
<th>ANOIKIS</th>
<th>AUTOPHAGY</th>
<th>WD</th>
<th>EXCITO-TOXICITY</th>
<th>ERYTHRO-POIESIS</th>
<th>PLT</th>
<th>CORNIFICATION</th>
<th>LENS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Genetic Program</strong></td>
<td>None</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td><strong>Membrane</strong></td>
<td>Lysed</td>
<td>intact PS exposure</td>
<td>intact PS exp.</td>
<td>intact PS exp.</td>
<td>intact</td>
<td>intact lipi-reassembly</td>
<td>intact lipi-reassembly</td>
<td>intact</td>
<td>intact lipi-reassembly</td>
<td>intact</td>
</tr>
<tr>
<td><strong>Organelles</strong></td>
<td>Lysed</td>
<td>intact</td>
<td>intact</td>
<td>intact</td>
<td>intact</td>
<td>crosslinked lipi-reassembly</td>
<td>lost</td>
<td>lost</td>
<td>lost</td>
<td>lost</td>
</tr>
<tr>
<td><strong>Mitos</strong></td>
<td>Blown</td>
<td>intact</td>
<td>intact</td>
<td>intact</td>
<td>intact</td>
<td>lost</td>
<td>lost</td>
<td>lost</td>
<td>lost</td>
<td>lost</td>
</tr>
<tr>
<td><strong>Nucleus</strong></td>
<td>chr.condens. DNA fragm.</td>
<td>intact</td>
<td>intact</td>
<td>intact</td>
<td>intact</td>
<td>lost</td>
<td>lost</td>
<td>lost</td>
<td>lost</td>
<td>lost</td>
</tr>
<tr>
<td><strong>Enzymes</strong></td>
<td>None</td>
<td>caspases</td>
<td>caspases</td>
<td>calpains</td>
<td>lysosomal beclin1</td>
<td>VPR</td>
<td>calpains</td>
<td>calpains</td>
<td>TG 1,3,5</td>
<td>TG</td>
</tr>
<tr>
<td><strong>Receptors</strong></td>
<td>Death Rec</td>
<td>caspases</td>
<td>caspases</td>
<td>calpains</td>
<td>lysosomal beclin1</td>
<td>VPR</td>
<td>calpains</td>
<td>calpains</td>
<td>TG 1,3,5</td>
<td>TG</td>
</tr>
<tr>
<td><strong>Regulators</strong></td>
<td>Bcl family IAP</td>
<td>caspases</td>
<td>caspases</td>
<td>calpains</td>
<td>lysosomal beclin1</td>
<td>VPR</td>
<td>calpains</td>
<td>calpains</td>
<td>TG 1,3,5</td>
<td>TG</td>
</tr>
</tbody>
</table>
```

---

**Classical Model**
- Type 1: Apoptosis
- Type 2: Autophagy
- Type 3: Necrosis

G. Mollino, P. Nicotera et al., *Cell Death and Differentiation*, 12 (2005)
Current cancer therapies, for example, chemotherapy, γ-irradiation, immunotherapy, or suicide gene therapy, primarily exert their antitumor effect by triggering apoptosis in cancer cells.

So far, no clear pattern has emerged between the level of apoptosis or proteins that regulate apoptosis and treatment response in most solid tumors.

Strategies Targeting the Intrinsic Pathway
- Bcl-2 Family Proteins
- Smac/Diablo Agonists
- Irradiation
Process of Autophagy

- Environmental stressors lead to isolation of double membrane-bound structures thought to be derived from the “phagophore”

- Membrane structures elongate and mature and MAP-LC3 is recruited to the membrane

- Elongated double membranes form autophagosomes and sequester cytosolic proteins and organelles

- Sequesteration requires ATP and microtubules

- Process can be inhibited by blocking ATP production or microtubule assembly

- The autophagosome fuses with the lysosome which proceeds with degradation

• Overexpression of beclin-1 (the yeast homolog of Atg6) induces autophagy in tumor cells and inhibits their tumorogenicity (acts as tumor supressor). Beclin-1 is regulated by BCL-2

• Other tumor supressor genes linked to autophagy include PTEN and tensin homologs

• Tamoxifen induces autophagic cell death of breast cancer cells through the oestrogen receptor and occurs through downregulation of AKT

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Treatment</strong></td>
<td><strong>Proposed target</strong></td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>Oestrogen receptor</td>
</tr>
<tr>
<td>Temozolomide</td>
<td>DNA</td>
</tr>
<tr>
<td>γ-Irradiation</td>
<td>DNA</td>
</tr>
<tr>
<td>Sodium butyrate and SAHA</td>
<td>HDAC</td>
</tr>
<tr>
<td>Hyperthermia</td>
<td>Unknown</td>
</tr>
<tr>
<td>Arsenic trioxide</td>
<td>Multiple targets (for example, mitochondria)</td>
</tr>
<tr>
<td>Resveratrol</td>
<td>Multiple targets (for example, oestrogen receptor and mitochondria)</td>
</tr>
<tr>
<td>Soybean B-group triterpenoid saponins</td>
<td>Unknown</td>
</tr>
<tr>
<td>Rapamycin</td>
<td>mTOR</td>
</tr>
</tbody>
</table>

HDAC, histone deacetylase; mTOR, mammalian target of rapamycin; SAHA, suberoylanilide hydroxamic acid.
Autophagy and Cancer

Markers of Autophagy

• MAP-LC3
  (Microtubule Associated Protein Light Chain 3)
  Homolog of the yeast Apg8 protein which is essential for formation of autophagosomes. Present as two isoforms (I&II). LC3-II Localizes to the limiting membranes of autophagosomes after processing.

• Monodansylcadaverine (MDC)
  lysomotrophic compound that has been shown to accumulate in autophagosomes (Biederbeck et al., 1995)
The ArrayScan VTI and High Content Screening (HCS)
Autophagy in Melanoma

<table>
<thead>
<tr>
<th></th>
<th>Hoechst 33342</th>
<th>MDC</th>
<th>LC3</th>
<th>MERGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>No tx</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
<tr>
<td>PBS (-) CaMg</td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
</tr>
<tr>
<td>As2O3</td>
<td><img src="image9.png" alt="Image" /></td>
<td><img src="image10.png" alt="Image" /></td>
<td><img src="image11.png" alt="Image" /></td>
<td><img src="image12.png" alt="Image" /></td>
</tr>
</tbody>
</table>
Autophagy in Melanoma

WM9 undergoes autophagy in response to starvation and arsenic trioxide as measured by an increase in cytoplasmic spot intensity.

Cells appear to be protected from autophagy in the presence of Ca++ and Mg++ cations.

LTR = Lysotracker Red
MDC = Monodansylcadaverine
LC3 = MAP LC3
Classes of Molecules That Initiate The Innate Immune Response – Signal 0

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.
Walter L Land, 2003
Classes of Molecules That Initiate The Adaptive Immune Response – Signal 3

IL-17A, IL-17E, ?

IL-12
IL-4
IFN-γ

IL-2
IL-4
IFN-γ

Th0 CD4+

IL23

IL17A, IL17E, ?

IL-10

IL-23

Th4

IL-12

Th1

IL-2, GM-CSF, IL-2, IFN-γ and TNF-β

IL-3, GM-CSF, IL-2, IFN-γ and TNF-β

IL-4, IL-5, IL-6, IL-10 and IL-13

TGF-β

T regulatory population

IL-10
### Classes of Molecules That Promote Continued Adaptive Response – Signal 0 – Environmental Stress

<table>
<thead>
<tr>
<th>T Helper</th>
<th>Signal 0</th>
<th>Inducer</th>
<th>Transcription Factor</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Th1</td>
<td>HMGB1, LPS, ?</td>
<td>IL12</td>
<td>T-bet</td>
<td>IFNγ</td>
</tr>
<tr>
<td>Th2</td>
<td>???</td>
<td>IL4</td>
<td>GATA3</td>
<td>IL4, IL5</td>
</tr>
<tr>
<td>Th3</td>
<td>???</td>
<td>IL10, TGFβ</td>
<td>FoxP3</td>
<td>IL10, TGFβ</td>
</tr>
<tr>
<td>Th4 [Th17]</td>
<td>↓?? Neut apoptosis</td>
<td>IL23</td>
<td>RORγ</td>
<td>IL17, IL6</td>
</tr>
</tbody>
</table>
The High Mobility Group Box-1 Protein, first identified in 1973.

- Serves as a DAMP (Danger Associated Molecular Pattern), linked to several important cellular processes ranging from release from necrotic cells to secretion by activated macrophages.
- Implicated in sepsis, ischemia-reperfusion, arthritis and cancer.

**Antagonist to B-Box activity**

**TNF stimulation**

**Stimulates transcription, binds chromatin, shields DNA-cisplatin from exonucleases**

---

**Intracellular HMGB1**

- Structural DNA binding protein
- Stabilizes Nucleosomes
- Transcriptional regulation

**Extracellular HMGB1**

- Neutrophil Chemotaxis
- Macrophage activation
- Dendritic cells maturation
- Vascular Leakage
- Acute Lung Injury
- Hepatic Injury
- Multiple Organ Failure
IL-23 promotes tumour incidence and growth

John L. Langowski¹*, Xueqing Zhang¹*, Lingling Wu¹, Jeanine D. Mattson¹, Taiying Chen¹, Kathy Smith¹, Beth Basham¹, Terrill McClanahan¹, Robert A. Kastelein¹ & Martin Oft¹
Type, Density, and Location of Immune Cells Within Human Colorectal Tumors Predict Clinical Outcome

Jerome Galon,† Anne Costes,† Patima Sanchez-Cabo, Amos Kirilovsky, Bernhard Mlecnik, Christine Lagorce-Pages, Marie Tosolini, Matthieu Camus, Anne Berger, Philippe Wind, Franck Zinzindohoué, Patrick Bruneval, Paul-Henri Cugnenc, Zlatko Trajanoski, Wolf-Herman Fridman,† Franck Pages,††

The role of the adaptive immune response in controlling the growth and recurrence of human tumors has been controversial. We characterized the tumor-infiltrating immune cells in large cohorts of human colorectal cancers by gene expression profiling and in situ immunohistochemical staining. Collectively, the immunological data (the type, density, and location of immune cells within the tumor samples) were found to be a better predictor of patient survival than the histopathological methods currently used to stage colorectal cancer. The results were validated in two additional patient populations. These data support the hypothesis that the adaptive immune response influences the behavior of human tumors. In situ analysis of tumor-infiltrating immune cells may therefore be a valuable prognostic tool in the treatment of colorectal cancer and possibly other malignancies.
# Classes of Molecules That Promote Innate Response – Signal 0 – Environmental Stress

<table>
<thead>
<tr>
<th>Sentinel</th>
<th>Stress</th>
<th>Antigen</th>
<th>Target</th>
<th>Signal 0</th>
<th>Signal 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>NK</td>
<td>Genomic Metabolic</td>
<td></td>
<td>MICA, MICB</td>
<td>HMGB1, LPS, ?</td>
<td></td>
</tr>
<tr>
<td>NKT</td>
<td>Cell Membrane</td>
<td>Glycolipid</td>
<td>CD1d</td>
<td>???</td>
<td></td>
</tr>
<tr>
<td>CD4</td>
<td>Cell membrane</td>
<td>13-20 Peptide</td>
<td>Class II</td>
<td>???</td>
<td></td>
</tr>
<tr>
<td>CD8</td>
<td>ER Stress</td>
<td>8-10 Peptide</td>
<td>Class I</td>
<td>↓?? Neut apoptosis</td>
<td></td>
</tr>
</tbody>
</table>
d(GpG) and d(ApG) adducts
Release of HMGB1 from Melphalan But not Oxaliplatin Treated Cells

HCT116 16 hours after treatment with Oxaliplatin

HCT 116 16 hours after treatment with Melphalan

Petar Popovic/Herb Zeh
Flow Cytometry Intracellular HMGB1/Histone H1

A: Tumor

B: Tumor

NP40: 0.013%

UV: 500 mJ
HMGB1 Release Following Immune Mediated Lysis?
HMGB1 Release Following Immune Mediated Lysis?
HCT-116 cells treated with chemotherapeutic agents

A

Control

B

Melphalan

C

Oxaliplatin

- **Green**: HMGB1
- **Red**: Actin
- **Blue**: Hoechst
HMGB1 Release Assessed Within An ELISA

Viability (MTT Absorbance O.D.)

Control = 1.505
Control = 2.841
Control = 1.339
Control = 1.278

Concentration (μg/ml)                  Time (Hrs)

Control
Melphalan (320μg/ml)
Oxaliplatin (160μg/ml)
Paclitaxel (80μg/ml)*
Western blot of supernatant derived from tumor cells incubated with chemotherapeutic agents [48 hours].
Intracellular nuclear staining following treatment with melphalan or oxaliplatin (WM2348 melanoma cell line)
Intracellular nuclear staining of HMGB1 diminishes following treatment with cells with LAK activity.
LAK Induces Intracellular HMGB1 Release

PBMC

PBMC + IL-2
Immune Mediated Lysis of Tumor Cells Release HMGB1

Tumor: 451Lu, 4h, 5x10^6/ml E/T=10/1
Immune Mediated Lysis of Tumor Cells Release HMGB1

FEM X (HLA-A2+)

MEL397 (HLA-A2-)

Immune Mediated Lysis of Tumor Cells Release HMGB1

Histone H1

-CTL +CTL

(A) (B)

(C) (D)

HMGB1 negative (%)

0

10

20

30

40

50

FEMEX MEL397

CTL

- +
Apoptosis and Necrosis
At The Event Horizon
Conclusions 1: Necrosis and Apoptosis and HMGB1

- DAMPs and HMGB1 are links between inflammation and necrotic cell death
- Apoptotic cell death causes sequestration of HMGB1 in the nucleus [phosphorylation of Histone 2B] – preliminary Marco Bianchi
- Platination of dsDNA causes sequestration of HMGB1 in the nucleus [dGpG and dGpA]
- Treatment of cancer [and arthritis] with platinums may succeed because of their ability to sequester HMGB1
Conclusions 2

- HMGB1 is indeed a pleiotrophic endokine – an endogenous danger signal promoting DC maturation and found in the serum of acute and chronic inflammatory states.
- HMGB1 synergizes with other cytokines in the mouse and man to promote acute immune reactivity [Schlepping or Chaperoning].
- HMGB1 in chronic inflammation may promote PDC suppression, promoting healing.
- Targeting HMGB1 with antibodies or soluble receptors may represent important strategies for treatment of microbial and inflammatory diseases.
Contributors

- Herb Zeh/Dave Bartlett/Pawel Kalinski/Zhong Shen Guo/Eric Dong/Petar Popovic/Nicole Schapiro
- Sanjiv Agarwala/John Kirkwood/Howard Edington/ Walter Storkus/Hassane Zarour/Lisa Butterfield
- Shaiv Dave, Scott Plevy

- Jukka Vakkila/Lina Lu/Mitchell Fink/Tim Billiar
- Richard DeMarco/David Montag/Norimasa Ito/Ramin Lotfi/Katie Horvath/Elisa Latorre/Tina Kilgore/Yuan Yu

- Anna Rubartelli
- Marco Bianchi
- Kevin Tracey
- Ann Marie Schmidt
- Theresa Ostberg, Helena Harris and Ulf Andersson
- Steven Rosenberg/John Wunderlich