iSBTc Workshop on Cancer and Inflammation: Promise for Biological Therapy October 30, 2008

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### Chronic Inflammatory Conditions Associated with Cancer

<table>
<thead>
<tr>
<th>Chronic inflammation</th>
<th>Associated cancer</th>
<th>Aetiological agent</th>
<th>Percent predisposed that progress to cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bronchitis</td>
<td>Lung cancer</td>
<td>Tobacco smoke</td>
<td>11–24</td>
</tr>
<tr>
<td>Gastritis</td>
<td>Gastric cancer</td>
<td><em>Helicobacter pylori</em></td>
<td>1–3</td>
</tr>
<tr>
<td>Cervicitis</td>
<td>Cervical cancer</td>
<td>Human papillomavirus</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Warts</td>
<td>Non-melanoma skin cancer</td>
<td>Ultraviolet light, human papillomavirus</td>
<td>Varies with skin pigment and solar intensity</td>
</tr>
<tr>
<td>Asbestososis</td>
<td>Mesotheloma</td>
<td>Asbestos fibres</td>
<td>10–15</td>
</tr>
<tr>
<td>Inflammatory bowel disease</td>
<td>Colorectal cancer</td>
<td>Gut pathogens, altered gut permeability</td>
<td>1*</td>
</tr>
<tr>
<td>Pancreatitis</td>
<td>Pancreatic cancer</td>
<td>Tobacco, genetic factors</td>
<td>≤10%*</td>
</tr>
<tr>
<td>Oesophagitis</td>
<td>Oesophageal cancer</td>
<td>Gastric acid, alcohol, tobacco</td>
<td>15</td>
</tr>
<tr>
<td>Sunburned skin</td>
<td>Melanoma, basal-cell carcinoma, squamous-cell carcinoma</td>
<td>Ultraviolet light</td>
<td>Varies with skin pigment and solar intensity, ≤99% of Caucasians</td>
</tr>
<tr>
<td>Hepatitis</td>
<td>Hepatocellular carcinoma</td>
<td>Hepatitis B virus, hepatitis C virus</td>
<td>10</td>
</tr>
<tr>
<td>Mononucleosis</td>
<td>Burkitt’s lymphoma, Hodgkin’s disease</td>
<td>Epstein–Barr virus</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Cholecystitis</td>
<td>Gall bladder cancer</td>
<td>Bacteria, gall bladder stones</td>
<td>1–2§</td>
</tr>
<tr>
<td>Cystitis</td>
<td>Bladder cancer</td>
<td>Gram-negative uropathogens, pelvic irradiation, carcinogens</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

*Per year. §In susceptible populations. †At cholecystectomy.

Inflammatory cells are present in all tumors
Current Focus Of Pathologic Evaluation

NEOPLASTIC CELLS:
Histological differentiation (architecture, nuclear grade, mitotic rate)
Biomarkers (e.g., hormone, growth factor receptors)
Gene expression profiling

STROMA:
“Desmoplastic stroma”
Presence of necrosis
Lympho/vascular invasion
Depth/extent of invasion

S. Demaria
Cancer Necrosis Correlates with Poor Prognosis

- Mesothelioma (Edwards, 2003) \( p=0.008 \)
- Renal-clear cell carcinoma (Cheville 2003; Tollefson 2007) \( p<.001 \)
- Colon carcinoma (Hunter, 1983)
- NSCLC (Swinson, 2003) \( p=0.0016 \)
- Breast (Gilchrist, 2003) \( p=0.0003; Kato, 2002 \) \( p=0.0068 \)
- Mucosal melanoma (Prasod, 2002) \( p=0.007 \)
- Melanoma (Balch, 2001)
- Sarcoma (Miyajima 2002; Gustafson 2003)
- Transitional Urothelial Carcinoma (Sang Eun Lee, 2007)
Type, Density, and Location of Immune Cells Within Human Colorectal Tumors Predict Clinical Outcome

Jérôme Galon,1,2† Anne Costes,1 Fatima Sanchez-Cabo,1 Amos Kirilovsky,3 Bernhard Mlecnik,2 Christine Lagorce-Pages,3 Marie Tosolini,1 Matthieu Camus1 Anne Berger,5 Philippe Wind,4 Franck Zinzindohoue,3 Patrick Brunet4 Paul-Henri Cugnenc,7 Zlatko Trajanoski,7 Wolf-Herman Fridman,1,7 Franck Pages1,7†

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 sample) 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.
State of the Science Sessions

• **Defining Inflammation**
  Michele Carbone, MD, PhD, *CRC of Hawaii*
  Sandra Demaria, MD, *New York University*

• **Genetic Polymorphisms and Factors which Modulate Inflammation and Cancer**
  Emad M. El-Omar, MB ChB, MD, *U. Aberdeen*
  Yen-Ching Karen Chen, ScD, SM, *National Taiwan University*

• **Animal Models of Cancer and Inflammation**
  Lisa M. Coussens, PhD, *UCSF*
  Michael Karin, PhD, *UCSD*
  [Eli Pikarsky, PhD *Hadassah*]
State of the Science Sessions

• Causes and Molecular Targets in Cancer and Inflammation
  Michael T. Lotze, MD, *UPCI*
  Giorgio Trinchieri, MD, *NCI*

• Current Clinical Evidence for Targeting Inflammation to Prevent Cancer
  Steven Dubinett, MD, *UCLA*
  Eva Szabo, MD, *NCI, NIH* [Jenny T. Mao]

• Novel Therapeutics and Clinical Trial Development to Rx Cancer
  George J. Weiner, MD, *University of Iowa*
  Arthur M. Krieg, MD, *Pfizer*
Defining Inflammation

1- Macrophages: how to differentiate TAM pro- and anti-tumor activities? Location within the tumor and/or markers?

2- MDSC: validate the use of IL-4Ra/? in human blood PBMC from cancer patients as a marker of immunosuppressive myeloid cells. How to evaluate the effects of pharmacological targeting of MDSC suppressive mechanisms in human tumors?
Defining Inflammation

3- Mast cells: how to differentiate activities? Location?; degranulation (tryptase, heparin)?

4- Eosinophils: how to define their role in cancer? Markers of function? Location?

5- DCs: DC-LAMP and CD83; IL-13 and/or pSTAT6?

6- TILs: CD4/CD8, granzymeB, and FoxP3 to obtain a more comprehensive and reliable prognostic indicator? Ready for prime time in colorectal, ovarian cancers and HCC? hepatocellular? Others? Role of Th17?
The T-cell ratio: pre-clinical data

Mouse melanoma model:
intratumoral ratio of Teff/Treg determines tumor rejection
Defining Inflammation

7- **Lymph Nodes**: Should sentinel lymph nodes (SLN) be analyzed immunologically?

8- **Methods for evaluation of prognostic/predictive parameters**: Oncotype DX equivalent for “immunological signature” of a tumor? Do morphology and IHC provide additional/different information? Predictor of response to immunotherapy?
The Crucial Role of T cells, NK Cells and DCs in the Tumor Microenvironment

No Substantial Difference in Number of Tumor Associate Leukocytes in Pediatric and Adult Cancers

Vakkila J, Jaffe R, Michelow M, Lotze MT. Pediatric cancers are infiltrated predominantly by macrophages and have a paucity of dendritic cells. Clinical Cancer Research, 2006 Apr 1;12(7):2049-54.
Marked Decrease in Dendritic Cells in Pediatric Cancers

Vakkila J, Jaffe R, Michelow M, Lotze MT. Pediatric cancers are infiltrated predominantly by macrophages and have a paucity of dendritic cells. Clinical Cancer Research, 2006 Apr 1;12(7):2049-54.
Comparable Number of Macrophages and Decreased DCs in Pediatric Cancer

Vakkila J, Jaffe R, Michelow M, Lotze MT. Pediatric cancers are infiltrated predominantly by macrophages and have a paucity of dendritic cells. Clinical Cancer Research, 2006 Apr 1;12(7):2049-54.
Genetic Polymorphisms and Factors which Modulate Inflammation and Cancer

1. TLR4, P2X7, IDO2, TRAIL, Perforin polymorphisms?
2. Pathway Analysis
3. *IL-1* gene cluster polymorphisms; tumor necrosis factor-\(\alpha\) (TNF-A) and *IL-10* polymorphisms
4. Include broader genotyping for known polymorphisms in clinical trials particularly of immunotherapeutic agents.
Animal Models

1. Can we identify cellular and molecular components that are common to all cancer-promoting inflammatory responses?

2. Innate immune cells directly and indirectly potentiate/limit cancer risk through the diversity of bioactive mediators they deliver to neoplastic tissues.

3. While the evidence for some mediators is strong (MMPs, cytokines, angiogenesis), for others there is less evidence (reactive oxygen and nitrogen species).
Animal Models

1. Define phenotypes and subtypes of hematopoietic cells (leukocytes, monocytes, mast cells, platelets).

2. Define the physiological roles of the immune cells and study the possible side effects resulting from neutralizing the pro-tumor properties of these cells utilizing immuno-depletion or pharmacologic inhibition strategies.

3. Better define the role of the various immune cells in the different stages of tumorigenesis.

4. Long-term usage of anti-inflammatory agents?

5. The adaptive immune system: etiology-, context- and organ-dependent roles [HCC, skin carcinogenesis examples]
Chemotherapy Induced Immunogenic Cell Death Does Not Correlate with Apoptosis/Necrosis
Calreticulin Exposure Correlates with Immunogenic Chemotherapy Induced Tumor Cell Death
Causes and Molecular Targets in Cancer and Inflammation

1. Role of Pathogen Associated Molecular Pattern Molecules – PAMPs \( \text{[H. pylori, EBV, HCV, HBV, HPV, polyoma virus, HHV8]} \)

2. Role of Damage Associated Molecular Pattern Molecules – DAMPs \( \text{[HMGB1, S100p and others, purine metabolites – ATP, uric acid, HSPs]} \)

3. Efforts to oxidize or neutralize factors
# 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

## Secreted molecules:
- DNA
  - Fibrinogen domain A
  - Surfactant protein A

## Matrix elements:
- Heparan sulfate
- Soluble hyaluronan
- Fibronectin
Prevention

• Chemoprevention refers to “the use of agents that can cause regression of existing preneoplastic lesions, prevent the progression of these lesions to cancer, prevent the development of new lesions” (Hong and Sporn, 1997).

• No recommended agents: interest in NSAIDs/COX2 inhibitors, steroids; ? others
Novel Strategies

A number of agents that would be expected to have a significant effect on inflammation within tumors are FDA approved (e.g. Bortezumib, Cytoxan and glucocorticoids) or under various stages of clinical development, yet we know little about the effect these treatments have on inflammation within tumors.

Start asking questions!
Autophagy promotes tumor cell survival and restricts necrosis, inflammation, and tumorigenesis

Kurt Degenhardt,1,2,3,10 Robin Mathew,1,9,10 Brian Beaudoin,1,3,10 Kevin Bray,2,3,4 Diana Anderson,3 Guanghua Chen,1,3,5 Chandreyee Mukherjee,1,3,5 Yufang Shi,6,9 Céline Gélinas,1,8,9 Yongjun Fan,1 Deirdre A. Nelson,5 Shengkan Jin,7,9 and Eileen White1,2,3,4,9,*

1Center for Advanced Biotechnology and Medicine, 679 Hoes Lane, Piscataway, New Jersey 08854

3:15 Cell Death

Metabolic Stress → Apoptosis → Autophagy → Necrosis

M → Recruitment/Cancer

BLNB-13
Life and Death

1] Inflammation
2] DAMPs
3] Redox-Anti-DAMPs; hyaluronan
4] Eosinophils
Rube Goldberg/Heath Robinson Devices – Life and Death

Phagocytosis of Apoptotic Neutrophils Regulates Granulopoiesis via IL-23 and IL-17

Matthew A. Stark,1 Yuqing Huo,2,4 Tracy L. Burcin,3 Margaret A. Morris,2,3 Timothy S. Olson,1,3 and Klaus Ley1,2,3,*
1Department of Molecular Physiology and Biophysical Sciences
2Department of Biomedical Engineering
3Cardiovascular Research Center
University of Virginia
Charlottesville, Virginia 22908
Effect of tumour cells killed by x-rays upon the growth of admixed viable cells.
Karolinska

Tumor Incidence

# The Four Step Cancer Model

## Chronic Inflammation and Cellular Necrosis Progress to Cancer

<table>
<thead>
<tr>
<th>Event</th>
<th>Chronic inflammation</th>
<th>Silencing of tumour-suppressor genes</th>
<th>Cell necrosis</th>
<th>Mutation of proto-oncogene(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mediators and mechanisms of action</td>
<td>STAT3, prostaglandins, IL-1β, IL-4, IL-10, TGF-β</td>
<td>Hypermethylation of CpG islands in promoter regions; enhanced by CI</td>
<td>CI-induced microthrombosis and micronecrosis results in release of HMGB1 and other necrotic factors</td>
<td>Autocrine growth or increased sensitivity to growth factors</td>
</tr>
<tr>
<td>Cells involved</td>
<td>T cells, macrophages, polymorphonuclear cells</td>
<td>Epithelial and stromal cells</td>
<td>Many normal and neoplastic cells distal and/or proximal to occluded arterioles and/or venules</td>
<td>Primarily epithelia, secondarily genetic and epigenetic changes in stromal cells</td>
</tr>
<tr>
<td>Consequences</td>
<td>Acute inflammatory reactions are downregulated; polarization to M2-type macrophages occurs; CI persists</td>
<td>Control of cell cycle or quality of DNA is lost; diminished apoptosis is promoted</td>
<td>Unscheduled cell death and reparative proliferation; increased probability of oncogenic mutations</td>
<td>Cancer</td>
</tr>
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