Cellular & Molecular Immunology

Cancer and the Immune System

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Lecture Overview

• Tumor “antigens” as targets of immune attack

• Potential host immune mechanisms that protect against development and growth of cancer cells

• Ways in which tumor cells circumvent or neutralize host immune mechanisms

• Immunotherapeutic strategies for treatment of cancer patients
What is Cancer?

a group of diseases characterized by uncontrolled growth and spread of abnormal cells
Cancer Factoids

- More than 1.2 million Americans develop cancer each year
- A new cancer is diagnosed every 30 seconds in the United States
- Since 2000, nearly 10 million new cancer cases have been diagnosed
- Cancer is the second leading cause of death after heart disease in the United States
Estimated New Cancer Cases*:
10 Leading Sites by Gender, US, 2002

30%  Prostate
14%  Lung & bronchus
11%  Colon & rectum
 7%  Urinary bladder
 5%  Melanoma of skin
 4%  Non-Hodgkin’s lymphoma
 3%  Oral cavity
 3%  Kidney
 3%  Leukemia
 2%  Pancreas
19%  All other sites

31%  Breast
12%  Lung & bronchus
12%  Colon & rectum
 6%  Uterine corpus
 4%  Ovary
 4%  Non-Hodgkin’s lymphoma
 4%  Melanoma of skin
 2%  Urinary bladder
 2%  Pancreas
 2%  Thyroid
20%  All other sites

*Excludes basal and squamous cell skin cancers and in situ carcinomas except urinary bladder.
What causes cancer?

Don’t know the exact causes, but evidence suggests contributing factors include:

Genetic influences – oncogenes

Environmental influences – (carcinogens) found in tobacco products, industrial pollutants, pesticides, fertilizers, etc.

Infectious organisms – some viruses and bacteria may be oncogenic

Suppression of immune protective mechanisms
How do cancer cells differ from normal cells?

Autonomous growth – failure to respond to regulatory growth controls

Ability to break away from the original tumor mass and migrate/grow in other tissues – Metastasis

Expression of “new” molecules (cell surface or intracellular) that are not present on normal cells

These new molecules (antigens) are potential targets for the immune response
Tumor rejection is an immune response

Mouse with chemical carcinogen-induced tumor

Remove tumor

Isolate CD8+ T cells

Adoptively transfer T cells into recipient of tumor transplant

Transplant tumor cells into original tumor-bearing mouse

No tumor growth

Transplant tumor cells into syngeneic mouse

Tumor growth

Eradication of tumor
## Tumor Antigens

<table>
<thead>
<tr>
<th>Tumor Antigens</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal host cell displaying multiple MHC-associated self antigens</td>
<td>No T cell response</td>
</tr>
<tr>
<td>Normal self protein</td>
<td></td>
</tr>
<tr>
<td>Mutated self protein</td>
<td>Various mutant proteins in carcinogen- or radiation-induced animal tumors; various mutated proteins in melanomas</td>
</tr>
<tr>
<td>Product of oncogene or mutated tumor suppressor gene</td>
<td>Oncogene products: mutated Ras, Bcr/Abl fusion proteins</td>
</tr>
<tr>
<td>CD8+ CTL</td>
<td>Tumor suppressor gene products: mutated p53 protein</td>
</tr>
<tr>
<td>Overexpressed or aberrantly expressed self protein</td>
<td>Overexpressed: tyrosinase, gp100, MART in melanomas. Aberrantly expressed: cancer-testis antigens (MAGE, BAGE)</td>
</tr>
<tr>
<td>CD8+ CTL</td>
<td></td>
</tr>
<tr>
<td>Oncogenic virus</td>
<td>Human papillomavirus E6, E7 proteins in cervical carcinoma; EBNA protein-induced lymphomas</td>
</tr>
</tbody>
</table>
### Table 17-1: Tumor Antigens

<table>
<thead>
<tr>
<th>Antigens From</th>
<th>Examples of Human Tumor Ags</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oncogenes &amp; tumor suppressor genes</td>
<td>Ras mutations (~10% human Ca); Her2/neu (breast &amp; other Ca)</td>
</tr>
<tr>
<td>Overexpressed normal genes</td>
<td>MART (melanomas; normally expressed in melanocytes)</td>
</tr>
<tr>
<td>Oncogenic viruses</td>
<td>Papilloma viruses (cervical Ca); EBNA-1 (EBV-assoc lymphomas);</td>
</tr>
<tr>
<td></td>
<td>SV40 T antigen (rodent tumors)</td>
</tr>
<tr>
<td>Oncofetal antigens</td>
<td>CEA (GI and other Ca; normal in liver &amp; during inflammation);</td>
</tr>
<tr>
<td></td>
<td>AFP (Alpha-fetoprotein)</td>
</tr>
<tr>
<td>Differentiation Ag</td>
<td>PSA; CD10 (ALL); Ig idiotypes (B lymphomas)</td>
</tr>
</tbody>
</table>
Oncofetal Antigens

Carcinoembryonic Antigen; Alpha fetoprotein
Monitoring serum CEA level in colon carcinoma

- Surgical removal of colon cancer
- Clinical detection of liver metastasis

CEA (ng/ml)

- Normal range

Time (days)
Normal vs. malignant B cells

- Malignant transformation can occur at any stage of development
- Malignant cells exhibit unregulated growth
- Daughter cells within the malignant clone express many of the same surface molecules as their normal counterparts
CD10 on normal and malignant B cells

<10% of PBMC are CD10+

100% of cells within the malignant clone are CD10+
Surface markers of human and murine peripheral B cells

Mouse:
- slg
- Igα
- Igβ
- I-A/I-E
- Ly2 [CD72]
- CR1
- CR2
- B220 [CD45]
- Ly-1 [CD5]
- FcγRII
- ME-R

Human:
- CD19
- CD20
- Igβ
- Igα
- slg
- CD40
- CD20
- CD40
- CR1 (CD35)
- CR2 (CD21)
- CD72
- CD5
- FcγRII (CD32)

MHC I
- MHC II
Flow cytometry and Fluorescence-Activated Cell Sorting (FACS)

Mixed population of cells labeled with fluorescent antibodies 1 (■) and 2 (●)

Nozzle

Laser

Analyzer controls charge on plates to vary deflection

Parallel plates to deflect flowing cells

Computer display

Fluor. 1 (red) positive
Double positive
Double negative
Fluor. 2 (green) positive
Immunophenotype of a patient with Chronic Lymphocytic (B cell) Lymphoma (CLL)

Positive for:
- CD5
- CD20
- CD19
- CD23 (FcR);
- kappa light chain

Negative for:
- CD3
- lambda light chain
<table>
<thead>
<tr>
<th>Stage of maturation</th>
<th>Stem cell</th>
<th>Pre-B cell</th>
<th>Immature B cell</th>
<th>Mature B cell</th>
<th>Activated B cell</th>
<th>Antibody-secreting cell</th>
</tr>
</thead>
</table>

**Diagram:**

- Extracellular space
- IgM
- Immunoreceptor tyrosine-based activation motif (ITAM)
- Plasma membrane
- Cytoplasm

*From Abbas, Lichtman, & Pober: Cellular and Molecular Immunology. W.B. Saunders, 1999, Fig. 9-3*
Southern blot analysis of Ig gene recombination

Ig DNA in uncommitted (embryonic) cell, nonlymphoid cell

Southern blot

6 kb

V1 V2 Vn J1J2 C

J region DNA probe

2.5 kb

V1 J1 J2 C

6 kb

1.5 kb

V1 J2

6 kb

1.5 kb

Ig DNA in committed cells of B lymphocyte lineage
Two DNA fragments containing \( J_h \) sequences that hybridize to the probe. These fragments are a consequence of rearrangement of the immunoglobulin heavy chain genes in this monoclonal B cell line.

DNA fragments from the unrearranged, or germline, configuration of the immunoglobulin heavy chain genes.

Background ‘smear’ due to hybridization of the probe to many DNA fragments corresponding to the many different rearrangements in a population of different B cells.
Ig expression during B lymphocyte maturation

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<th>Stage of maturation</th>
<th>Stem cell</th>
<th>Pre-B cell</th>
<th>Immature B cell</th>
<th>Mature B cell</th>
<th>Activated B cell</th>
<th>Antibody-secreting cell</th>
</tr>
</thead>
</table>

**Immunoglobulin structure**

- Antigen-binding site
- Fc receptor and complement binding sites
- Disulfide bond
- Carbohydrate
- Heavy chain
- Light chain
- Ig domain
Electrophoresis patterns

Normal

Multiple Myeloma
Concept of Immunosurveillance
(Lewis Thomas & McFarland Burnet; 1959)

Immune system functions to recognize and destroy clones of malignant cells before they develop into clinically relevant tumors. (T cells)

Based on this concept, one would expect a higher incidence of cancer in people who manifest T cell immunodeficiencies.

However, there is not an inordinately higher frequency of common cancers (breast, prostate, colon, lung) associated with T cell immunodeficiency states.
## Immunodeficiency (ID) and Cancer

<table>
<thead>
<tr>
<th>Cause of ID</th>
<th>Type of Cancer</th>
<th>Virus involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inherited</td>
<td>B cell lymphoma</td>
<td>EBV</td>
</tr>
<tr>
<td>Immuno-suppression for transplants or due to AIDS</td>
<td>B cell lymphoma</td>
<td>EBV</td>
</tr>
<tr>
<td></td>
<td>Cervical</td>
<td>Papilloma</td>
</tr>
<tr>
<td></td>
<td>Skin</td>
<td>Papilloma</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>Hepatitis B</td>
</tr>
<tr>
<td></td>
<td>Kaposi’s Sarcoma</td>
<td>KSHV-8</td>
</tr>
<tr>
<td>Infectious Disease; Malaria</td>
<td>B cell (Burkitt’s) lymphoma</td>
<td>EBV</td>
</tr>
</tbody>
</table>
Role of EBV in tumorigenesis

immunity to EBV in normal individuals

CR2 (CD21) = EBV Receptor

infection

infected B cells killed

B

B

Tc

extracellular viruses eliminated

TH

help

EBV antigen

APC

extracellular viruses eliminated

B

B
Role of EBV in tumorigenesis

Immunosuppression and EBV

- No response to antigen
- Virus replication and infection
  - Mitogenic effect of virus causes proliferation of normal B cells
  - Chromosomal translocation creates B-cell tumour
Potential Host Immune Mechanisms Against the Development of Cancer

- Anti-tumor antibodies
- Tumor-specific Cytotoxic T Lymphocytes (CTL)
- Natural Killer (NK) cells (resting or activated)
- Activated Macrophages
Potential Host Immune Mechanisms: Antibody response against tumor antigens

From Abbas, Lichtman, & Pober: Cellular and Molecular Immunology. W.B. Saunders, 1999, Fig. 9-2
Potential Host Immune Mechanisms: Stimulation of tumor specific Cytotoxic T Lymphocytes (CTL)

Fig 17-3
Potential Host Immune Mechanisms:
Resting and cytokine activated NK cells
NK progenitor → NK → LAK

IFNY/IL-2

Killing

Tumour lines

Fresh tumour isolates (in vivo renal carcinoma and melanoma)
Potential Host Immune Mechanisms: Resting and cytokine activated macrophages
Antibody Dependent Cell-mediated Cytotoxicity (ADCC)

ADCC Effector Cells = NK cells, macrophages, eosinophils
Paradox: Tumor growth can occur in spite of immune reactivity
Genetic Factors
Innate & Adaptive Immunity

Immunological escape

Tumour destruction

Tumour Escape Mechanisms

Tumour growth
Tumor escape mechanisms:

Location  Location  Location

Diagram showing the circulatory system with various blood vessels and lymph nodes. The diagram includes labels for venous blood, arterial blood, lymph, lymph node, and various cells (naive T cell, activated effector or memory T cell). The heart, aorta, superior vena cava, and thoracic duct are also labeled.
Tumor escape mechanisms:
Not all tumor antigens are created equal
(i.e., equally immunogenic)
MHC molecules present peptide antigens
Fig 17-4: Tumor Escape Mechanisms

- T cell recognition of tumor antigen leading to T cell activation
- Failure to produce tumor antigen
  - Antigen-loss variant of tumor cell
  - Lack of T cell recognition of tumor

- Mutations in MHC genes or genes needed for antigen processing
  - Class I MHC-deficient tumor cell
  - Lack of T cell recognition of tumor

- Production of immunosuppressive proteins
  - Inhibition of T cell activation
  - Immunosuppressive cytokines
Tumor escape mechanisms:
Failure to express co-stimulatory molecules

The most common cancers (breast, prostate, lung, colon) do not express co-stimulatory molecules
The major goals of immunotherapy:

• to be used with other forms of treatment to seek out and destroy tumor cells at metastatic sites

• to identify tumor antigens

• to stimulate the patient’s own immune system to recognize antigens on tumor cells and mount effective mechanisms to destroy the tumor cells

• to be effective with minimal side effects
# Immunotherapy Against Cancer

<table>
<thead>
<tr>
<th>Active</th>
<th>Non-specific</th>
<th>Specific</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BCG; C. parvum</td>
<td>Tumor vaccines</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Passive</th>
<th>Non-specific</th>
<th>Specific</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cytokines</td>
<td>Antibodies coupled to drugs, toxins, radioisotopes; CTL</td>
</tr>
</tbody>
</table>

Active immunotherapy requires the recipient to respond to the agent being administered.
<table>
<thead>
<tr>
<th>Type</th>
<th>Preparation</th>
<th>Clinical Trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Killed tumor vaccine</td>
<td>Cells</td>
<td>Melanoma; Colon Ca</td>
</tr>
<tr>
<td></td>
<td>Lysates</td>
<td>Melanoma</td>
</tr>
<tr>
<td>Purified tumor antigen</td>
<td>Melanoma Antigen</td>
<td>Melanoma</td>
</tr>
<tr>
<td></td>
<td>Heat Shock Protein</td>
<td>Melanoma; Renal Ca</td>
</tr>
<tr>
<td>APC-based Vaccines</td>
<td>DC + tumor Ag</td>
<td>Melanoma; NHL; Prostate</td>
</tr>
<tr>
<td></td>
<td>DC + tumor Ag gene</td>
<td>Various carcinomas</td>
</tr>
<tr>
<td>DNA Vaccines</td>
<td>Imm w tumor Ag encoding plasmids</td>
<td>Melanoma</td>
</tr>
<tr>
<td>Viral Vectors</td>
<td>Adeno or Vaccinia with tumor Ag</td>
<td>Melanoma</td>
</tr>
</tbody>
</table>
Fig 17-5: Tumor Vaccines

A. Vaccinate with tumor-antigen pulsed dendritic cell

- Dendritic cells pulsed with tumor antigens
- CD8+ T cell
- Activation of tumor-specific T cells

B. Plasmid expressing cDNA encoding tumor antigen

- Vaccinate with DNA or transfected dendritic cell
- Dendritic cells transfected with plasmid expressing tumor antigen
- APC producing tumor antigen
- CD8+ T cell
- Activation of tumor-specific T cells
<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Clinical Trials</th>
<th>Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-2</td>
<td>Melanoma; Renal; Colon (&lt;15% response)</td>
<td>Vascular leak; shock; pulmonary edema</td>
</tr>
<tr>
<td>TNF</td>
<td>Melanoma; Sarcoma</td>
<td>Septic Shock Syndrome</td>
</tr>
<tr>
<td>IL-12</td>
<td>Phase I Toxicity; Melanoma and others</td>
<td>Abnormal liver function</td>
</tr>
<tr>
<td>IL-6</td>
<td>Renal (no benefit seen)</td>
<td>Fever; abnormal liver; CNS toxicity; hypotension</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>Routine use to promote BM recovery in patients</td>
<td>Bone pain</td>
</tr>
</tbody>
</table>
Fig 17-6: Cytokine Gene-transfected Tumor Cells

A. Vaccinate with tumor cell expressing costimulators or IL-2. Tumor cell transfected with gene for lymphocyte costimulator (e.g., B7) or IL-2. B7-expressing tumor cell stimulates tumor-specific T cell. IL-2 enhances proliferation and differentiation of tumor-specific T cells.

B. Vaccinate with tumor cell expressing GM-CSF. Tumor cell transfected with gene for GM-CSF. GM-CSF promotes recruitment and maturation of dendritic cells. Dendritic cell ingests, processes, and presents tumor antigens to tumor-specific T cells. APC

GM-CSF

Phagocytosed tumor cell

CD8+ T cell

Activation of tumor-specific T cells
## Table 17-5: mAbs for Cancer Therapy

<table>
<thead>
<tr>
<th>Specificity</th>
<th>Form of mAb used</th>
<th>Clinical Trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Her-2/Neu</td>
<td>Humanized mouse mAb</td>
<td>Breast Ca (approved for clinical use)</td>
</tr>
<tr>
<td>CD20</td>
<td>Humanized mouse mAb</td>
<td>B cell lymphoma</td>
</tr>
<tr>
<td>CD10</td>
<td>Humanized mouse mAb</td>
<td>B cell lymphoma</td>
</tr>
<tr>
<td>CEA</td>
<td>Humanized mouse mAb</td>
<td>GI and lung cancers</td>
</tr>
<tr>
<td>CA-125</td>
<td>Mouse mAb</td>
<td>Ovarian cancer</td>
</tr>
<tr>
<td>GD3 Ganglioside</td>
<td>Humanized mouse mAb</td>
<td>Melanoma</td>
</tr>
</tbody>
</table>
B-Cell Lymphomas Express Several Antigens That Can Be Targeted

CD20 Is Not Expressed on Stem Cells or Plasma Cells

BEXXAR: Characteristics

Tyrosine Tyrosine

Tositumomab
(Murine IgG2a)

CD20 Binding

Tyrosine label

γ and β-radiation

\(^{131}I\)
Tumors can develop resistance to treatment

Downregulation or mutation of surface molecules

Resistance to continued treatment

Anti-CD20 mAb

Generation of tumor-specific CTL clones

1. Surgically resect tumor
2. Purify mononuclear cells from tumor site
3. Tumor cells
4. Coculture mononuclear cells and melanoma cells
5. Isolate and clone activated CD8+ CTLs
6. Patient's mononuclear cells
7. Melanoma cell line