Hallmarks of Cancer

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What is Normal?

Dependence on growth factors
  – Cell and tissue specific signals
  – Loss of these signals leads to apoptosis

• Anchorage dependant proliferation
  – Requires interaction of transmembrane proteins (integrins) with components of the ECM

• Contact inhibition
  – Contact with other cells inhibits proliferation and movement

• Limited proliferative capacity
  – Normal somatic cells have a limited number of divisions before entering senescence
Acquired Capabilities of Cancer

- Self-sufficiency in growth signals
- Insensitivity to anti-growth signals
- Evading apoptosis
- Sustained angiogenesis
- Tissue invasion & metastasis
- Limitless replicative potential
Hallmark #1: Self-Sufficiency in Growth Signals: Oncogenes

- Originally coined as a genetic term to describe any gene capable of causing cancer
  - Later came tumor suppressors – loss of function genes that can cause cancer

- Oncogenes refers to genes that contribute to cancer in a gain-of-function manner

- Proto-oncogenes are the normal genes
Tumor-Suppressor Genes Vs Oncogenes

RESULT

NORMAL CELL PROLIFERATION

EXCESSIVE CELL PROLIFERATION

EXCESSIVE CELL PROLIFERATION
Routes to the Genesis of an Oncogene

- Translocation
- Over-expression

Figure 23–27. Molecular Biology of the Cell, 4th Edition.
Myc Regulates Proteins Involved in Cell Cycle Progression

- Myc regulates the expression of cyclin D gene, leading to increased cyclin D.
- Myc increases SCF subunit gene expression, resulting in increased p27 degradation.
- Myc increases E2F gene expression, resulting in increased E2F synthesis.

Cyclin D and p27 degradation result in G1-Cdk activation (cyclin D–Cdk4) and G1/S-Cdk activation (cyclin E–Cdk2), respectively.

Rb phosphorylation leads to increased E2F activity, facilitating entry into S phase.
Hallmark #2: Insensitivity to Negative Signals: Tumor Suppressor Genes

- Tumor suppressor genes are altered by inactivating mutations and this can lead to cancer
  - Point mutations
  - Deletion of regions of chromosomes
  - LOH
  - Altered methylation of the promoter
Classical Tumor Suppressor Genes

- Retinoblastoma gene (Rb)
  - Germline mutations in Rb and one acquired somatic mutation

**NORMAL, HEALTHY INDIVIDUAL**

- Occasional cell inactivates one of its two good Rb genes

**HEREDITARY RETINOBLASTOMA**

- Inherited mutant Rb gene
- Occasional cell inactivates its only good Rb gene copy
- Excessive cell proliferation leading to retinoblastoma

**RESULT: NO TUMOR**

**RESULT: MOST PEOPLE WITH INHERITED MUTATION DEVELOP TUMOR**
The Rb Pathway and the Cell Cycle
Classic Tumor Suppressor Genes

- **p53 gene**
  - 60-70% of all cancers have a p53 mutation

- Loss of both alleles or a dominant negative allele

An important consequence of p53 loss is a sharply elevated mutation rate, as well as genetic instability. Both are due to the inability to arrest the cell cycle in response to DNA damage.
p53 Mutation or Loss Plays a Dominant Role in Cancer
Hallmark #3: Evasion of Apoptosis

- Apoptosis is programmed cell death
  - Apoptosis is programmed cell death, in which morphological nuclear changes and DNA fragmentation occur. The nuclear changes are nuclear blebbing to form micronuclei, and condensation of chromatin. Other features include loss of intercellular contact, vacuolation, and a relative conservation of cellular organelles.

  - Necrosis, the other major type of cell death occurs as a result of cytoplasmic membrane damage, ion release and osmotic lysis with little nuclear or DNA damage until relatively late in the process.

- Damaged cells are effectively removed by this mechanism
  - This is a mechanism by which cells that have oncogenic mutations are removed
  - Is a critical defense against cancer
Programmed Cell Death is an Essential Aspect of Animal Life
Evasion of Apoptosis

- Death receptors transmit signals leading to apoptosis
  - FAS ligand and FAS receptor
  - TNF-α and TNF-αR1
  - Decoy receptors that don’t signal can promote survival

- Intracellular proteins that monitor DNA damage
  - p53

- Pro-survival factors
  - Bcl-2 family of proteins
Myc Suppresses Bcl-X\textsubscript{L} Expression in Pre-B Cells

Bcl-X\textsubscript{L}  
Bax  
Bcl-2  

wild-type

4-HT (hrs)  
0  6  12  24

Hsp  
ER  

Myc  

Max  

CACGTG
Myc Suppresses Bcl-2 or Bcl-\(X_L\) Expression in a Cell Context-Specific Fashion
Apoptosis

c-Myc

Bcl-X

Bcl-2

Apoptosis
Hallmark #4: Acquisition of Limitless Proliferative Capacity

- Cells have a finite lifespan and limited ability to replicate
  - Due to chromosome shortening
  - Ends of chromosomes are called telomeres (hexamer repeats - TTAGGG)

- Hayflick limit: approximately 50-80 doublings
  - Cells reach replicative senescence

- Inactivating pRb or p53 extends lifespan 30 doublings

- Rare mutations lead to immortalization
  - Activation of telomerase
Hallmark #5: Sustained Angiogenesis

• All tumors require a blood supply to grow to a significant size

• Pro-angiogenic factors such as VEGF, FGF1 and FGF2 are activated in tumors and signal endothelial cell proliferation and growth of blood vessels.
Angiogenesis and Vascular Endothelial Cells

Blood vessel

Vascular endothelial cells
Angiogenesis

PC/SMC functions:
- EC proliferation, migration
- EC differentiation, survival, quiescence
- vasomotion
- permeability, barrier, scavenger
- ECM production, integrity, hemostasis

Fig. 3 VEGF initiates assembly of endothelial cells (EC), PDGF-BB recruits pericytes (PC) and smooth muscle cells (SMC), whereas angiopoietin-1 (Ang1) and TGF-b1 stabilize the nascent vessel. Angiopoietin-2 (Ang2) destabilizes the vessel, resulting in angiogenesis in the presence of angiogenic stimuli, or in vessel regression in the absence of endothelial survival factors.
Angiogenesis and Regulatory Proteins

Concentration of Angiogenesis Inhibitors

- Inhibitors high
- Activators low

Blood vessel

Rare cell division

- Inhibitors low
- Activators high

Frequent cell division
# Anti-Angiogenic Factors

<table>
<thead>
<tr>
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<tr>
<td>Angiostatin</td>
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<td>CD59 complement fragment</td>
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<td>Endostatin</td>
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<tr>
<td>Fibronectin fragment</td>
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<td>Interferon inducible protein-10</td>
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<td>Platlet factor 4</td>
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<td>Prolactin (16kDa fragment)</td>
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<td>Interferon-α, -β, -γ</td>
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<td>Retinoids</td>
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<td>TSP-1</td>
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<td>Vasculostatin</td>
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Pro-Angiogenic Factors

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<td>Granulocyte-CSF</td>
<td>HGF</td>
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<tr>
<td>IL-8</td>
<td>VEGF</td>
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<tr>
<td>IL-6</td>
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Angiogenesis: A Delicate Balance

IL-8

bFGF

bFGF

VEGF

Angiostatin

Angiostatin

Ang1

Tsp-1

Ang1

Tsp-1

Endostatin

Angiogenesis

Angiogenesis
The Angiogenesis Signaling Cascade

Cancer cell

VEGF (or bFGF)

Receptor

Endothelial Cell

Transcription

Proteins stimulate new endothelial cell growth
Angiogenesis and VEGF in Cancer

Association between VEGF and angiogenesis, malignancy and metastasis has been demonstrated

• Many pro- and anti-angiogenic cellular factors regulate angiogenesis

• Research suggests that VEGF is a potent and predominant factor in this process
Progression of Cancer

Initiation

Promotion

Diffusion

Angiogenesis

Perfusion

Invasion

J Folkman 1974

IJ Fidler 2002
What Is Tumor Angiogenesis?

- Small localized tumor
- Blood vessel
- Signaling molecule (i.e. VEGF)

Angiogenesis

- Tumor that can grow and spread
Angiogenesis and Cancer

Old Theory

New Theory

Vessel dilation

Angiogenesis
Structure and Function of Tumor Vessels

• Abnormal architecture: tumor vasculature is highly disorganized
  - Vessels are tortuous and dilated with uneven diameter, excessive branching and shunts

• High vascular permeability: walls have numerous ‘openings’ (endothelial fenestrae, vesicles and transcellular holes), widened inter-endothelial junctions, and a discontinuous or absent BM
Normal vs Tumor Vasculature
Disorganized and Numerous

McDonald & Choyke Nat Med 2003
Hallmark #6: Tissue Invasion and Metastasis

- Cell-Extracellular Matrix Interactions are altered
  - Changes in structural proteins (e.g. integrins)
  - Changes in enzymes (signaling enzymes, proteases, etc.)
  - Not as relevant to hematologic tumors

- Result: Increased Migration and invasion (critical steps in metastasis)
Tissue Invasion and Metastasis

- **Primary Tumour**
- **Proliferation/angiogenesis**
- **Detachment/invasion**
- **Embolism/circulation**
- **Extravasation**
- **Adherence to vessel wall**
- **Arrest in organs**
- **Transport**
- **Metastasis**

- **Establishment of a microenvironment**
- **Proliferation/angiogenesis**
<table>
<thead>
<tr>
<th>Component</th>
<th>Acquired Capability</th>
<th>Example of Mechanism</th>
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<tbody>
<tr>
<td></td>
<td>Self-sufficiency in growth signals</td>
<td>Activate H-Ras oncogene</td>
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<td>Insensitivity to anti-growth signals</td>
<td>Lose retinoblastoma suppressor</td>
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<td></td>
<td>Evading apoptosis</td>
<td>Produce IGF survival factors</td>
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<td>Limitless replicative potential</td>
<td>Turn on telomerase</td>
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<td>Sustained angiogenesis</td>
<td>Produce VEGF inducer</td>
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<td>Tissue invasion &amp; metastasis</td>
<td>Inactivate E-cadherin</td>
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</table>
E2F1 and its role in Myc-induced lymphomagenesis

c-Myc and its role in angiogenesis and tumorigenesis
CANCER

Homeostasis

Proliferation → Differentiation

Proliferation → Apoptosis

CANCER

Proliferation

Differentiation

Apoptosis
Burkitt’s Lymphomas, other human lymphoid malignancies

AML, Lung, Breast, Colon, Brain, & Prostate cancers

Breast, Ovarian, & Colon carcinomas

• 70% of all cancers have deregulated Myc
Structure and Function of Myc Family Members

- **c-Myc**
  - MB1
  - MB2
  - TAD
  - Repression
  - NLS
  - Zip
  - HLH
  - Length: 439

- **N-Myc**
  - MB1
  - MB2
  - TAD
  - Repression
  - NLS
  - Zip
  - HLH
  - Length: 462

- **L-Myc**
  - MB1
  - MB2
  - Repression
  - NLS
  - Zip
  - HLH
  - Length: 364
Transcriptional Regulation by Myc
The c-Myc-ARF-p53 Pathway

- Myc
- ARF
- Mdm2
- p53

Cell Cycle Arrest

Apoptosis
Both ARF and p53 Mediate Myc-Induced Apoptosis in B-Cells
The Eμ-\textit{myc} Transgenic Mouse

Peripheral Pre-B cells

BM Pre-\& B-cells

Clonal B-cell Lymphoma

DEATH

2 Weeks \rightarrow 2 Months \rightarrow 3-5 Months
Eμ-myc Lymphomas Express High Levels of \textit{ODC} & \textit{VEGF} & are Highly Vascularized

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{figure}
\caption{Expression levels of VEGF and \textit{β-actin} in Eμ-myc and WT samples.}
\end{figure}
The ARF-p53 Pathway is Constitutively Active in Eμ-myc Bone Marrow
The ARF-Mdm2-p53 Pathway Harnesses Myc-Induced Lymphomagenesis

- **ARF^{+/+} (n=31)**
  - 24% ARF deletes
  - 28% mutant p53

- **ARF^{+-} (n=85)**
  - 80% ARF deletes
  - No p53 mutants

- **ARF^{-/-} (n=20)**
  - No p53 Mutants
The c-Myc-ARF-p53 Pathway

Myc

ARF

Mdm2

p53

E2f1

Apoptosis

Cell Cycle Arrest

Leone et al. 2001
Myc-Induced Apoptosis is Independent of $E2f1$ in MEFs

Myc

Hsp ER

ER

+ 4-HT

Max

CACGTG

% Viability

Time (hrs)

WT

$E2f1^{-/-}$

WT/Myc-ER

$E2f1^{-/-}$/Myc-ER

0 3 6 12 24

Myc-ER

20 40 60 80 100

% Viability
Myc Activates the ARF-p53 Pathway Independent of \textit{E2f1}
**E2f1 Loss Impairs** Myc-Induced Lymphomagenesis

- **E2f1** loss impairs Myc-induced lymphomagenesis.

The survival graph shows:
- **E2f1**+/+(n=22)
- **E2f1**+/- (n=48)
- **E2f1**-/- (n=25)

Statistical significance: \( P < 0.001 \)
Expression of p53, p19\textsuperscript{Arf} and E2F1 in Eu-\textit{myc} lymphomas
The ARF-p53 Pathway Is Frequently Inactivated in Myc-Induced Tumors

- p53 mutation
- ARF delete
- Mdm2 Overexpression
Myc-Induced Death is Independent of $E2f1$

**Ex vivo**

- Wild Type
- $E2f1^{+/+}$, Myc
- $E2f1^{+/-}$, Myc
- $E2f1^{-/-}$, Myc

**In vivo**

- $E2f1^{+/+}$, Myc
- $E2f1^{+/-}$, Myc
- $E2f1^{-/-}$, Myc

Bar graph showing the percentage of sub-G1 cells in different genotypes: $E2f1^{+/+}$, $E2f1^{+/-}$, and $E2f1^{-/-}$ with and without Myc induction.
The c-Myc-ARF-p53 Pathway

Myc → ARF → Mdm2 → p53

E2f1 → [X] → ? Cell Growth

→ Apoptosis
*E2f1* Loss Impairs Myc-Induced B cell Growth *Ex vivo*

![Graph showing % S phase for different *E2f1* and *Eμ-myc* genotypes.](image)
**E2f1 Loss Decreases Myc-Induced Cell Growth In B Cells In Vivo**

**Bone Marrow**

- **WT**
- **Eμ-myc**

**Spleen**

- **WT**
- **Eμ-myc**
• Loss of E2f1 impairs Myc-induced lymphomagenesis

• E2f1 loss is impairing Myc-induced cell growth, but not Myc-induced apoptosis
p27 Loss Enhances Myc-Induced Lymphomagenesis

Martins and Berns, EMBOJ 21:3739-48 2002
*E2f1* loss Blocks Myc-Mediated Suppression of p27^Kip1
E2f1 is Required for Myc-Induced Downregulation of p27Kip1

A. E2f1 +/-

<table>
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<tr>
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<th>Myc-ER</th>
<th>E2f1-ER</th>
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<td>4-HT (hr)</td>
<td>0</td>
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<td>12</td>
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p27 Actin

B. E2f1 -/-

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p27 Actin
Myc-Mediated Proliferation and Lymphomagenesis, But Not Apoptosis Is Compromised by \textit{E2f1} Loss
Acknowledgements

• Jennifer Old
• Chuck Sherr
• Martine Roussel