The Hallmarks of Cancer: 
An Introduction to the Molecular Biology of malignancy

A. Eliopoulos

Cancer arises when a cell, for a variety of reasons, escapes from the normal constraints placed on its growth and begins to divide in an unregulated fashion.

Cancer

Major social problem:
1996: 10 million new cancer cases worldwide, 6 million deaths
2020: 20 million new cancer cases worldwide, 10 million deaths (predicted)
The causes of cancer

• Environment
  Chemical carcinogens (i.e. tobacco smoke, asbestos), Biological carcinogens (i.e. viruses, bacteria)
  Physical carcinogens (i.e. radiation)

• Metabolic polymorphisms (SNPs affecting enzymes involved in carcinogen metabolism or immune response).

• Genetic pre-disposition

Metabolic enzyme polymorphisms

Phase I enzymes are involved in the activation (usually oxidation) of carcinogens, i.e. cytochromes P450 activate nitrosamines.

Phase II enzymes are involved in the inactivation of carcinogens, i.e. glutathione S-transferases and N-acetyltransferase. SNPs which reduce N-acetyltransferase activity towards the chemical arylamines are linked to pre-disposition to bladder cancer.
The hallmarks of cancer:
An introduction to the Molecular Biology of Malignancy

The facts:
- Cancer cells frequently contain 3-7 somatic mutations per cell.
- Benign tissue surrounding the tumor frequently contains some but not all the mutations found in the malignant tissue.
- Certain genes have a higher probability of mutating in a given tissue and stage of disease progression.

The questions:
- Why so many mutations are needed for oncogenesis?
- What is the interplay between malignant and normal cells?
- Which genes have higher mutation probability and what is their role?

Alterations in three types of genes cause cancer

1. Oncogenes
2. ‘Gatekeeper’ genes
3. ‘Caretaker’ or ‘Mutator’ genes

Tumor suppressor genes

Gatekeeper proteins prevent unwanted cell growth by eliminating potential cancer cells
Caretaker proteins protect the genome from accumulating oncogenic mutations.

Mutations, mutations, mutations...
The unifying principle of tumour development

<table>
<thead>
<tr>
<th>Damage</th>
<th>Events per cell per day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single-strand breaks</td>
<td>55,000</td>
</tr>
<tr>
<td>Deaminations</td>
<td>13,000</td>
</tr>
<tr>
<td>Depurinations</td>
<td>900</td>
</tr>
<tr>
<td>Guanine-O6 methylation</td>
<td>3100</td>
</tr>
<tr>
<td>Cytosine deamination</td>
<td>200</td>
</tr>
<tr>
<td>Thymine glycol</td>
<td>270</td>
</tr>
<tr>
<td>Thymidine glycol</td>
<td>70</td>
</tr>
<tr>
<td>Hydroxymethyluracil</td>
<td>500</td>
</tr>
<tr>
<td>Guanine-8 oxygenation</td>
<td>400</td>
</tr>
<tr>
<td>Interstrand cross-link</td>
<td>8</td>
</tr>
<tr>
<td>Double-strand break</td>
<td>9</td>
</tr>
<tr>
<td>DNA-protein cross-link</td>
<td>unknown</td>
</tr>
</tbody>
</table>
Ethylmethane Sulfonate (EMS) acetylation of guanine.

Mutations & DNA repair

- DNA polymerase proofreading activity
- DNA mismatch repair
- Nucleotide excision repair
- Recombination repair
- Base excision repair

Dietary components
Mutations & DNA repair

- DNA polymerase proofreading activity
- DNA mismatch repair
- Nucleotide excision repair
- Recombination repair
- Base excision repair

DNA mismatch repair (MMR)

hMutSα complex (MSH2, MSH6, MLH1 & PMS2)

Single bp MMR

Insertion/deletion Loop repair

Slippage between the template and replicating strands
DNA mismatch repair (MMR)

hMutSα complex (MSH2, MSH6, MLH1 & PMS2)

hMutSβ complex (MSH2, MSH3, MLH1 & hMLH3)

What if this repair system fails?

Hereditary Non-Polyposis Colon Cancer: the most common cancer predisposition syndrome
- 60% hMLH1 mutations
- 35% hMLH2 mutations

Colon cancer without related family history:
- 15% display MLH1 promoter hypermethylation and gene inactivation

Loss of MMR function renders tumor cells resistant to chemotherapy

Therefore...
- DNA repair is required for normal cell and tissue homeostasis
- Failure to repair the damage leads to cancer.

HOW?

Mutations in DNA damage-response signaling pathways ("Mutator genes")

Mutator Phenotype

Mutations in Cancer genes

Genomic instability

Mutations which increase Mutation rates
Genomic instability & cancer

- Genomic instability implies an abnormally high rate of genomic alterations.
- Observed early in carcinogenesis, i.e. benign tumors and variation and extent increases as tumors progress towards malignancy.
- Because DNA damaging agents do not target particular sequences, it is likely that a vast number of mutations are generated early in malignancy and there is selection for those mutations which are rate-limiting for tumor formation.
- 2 types: Microsatellite instability & chromosome instability

Microsatellite instability & cancer

- 'slippage' between the template and replicating strands

Replication slippage, mutator pathway and MSI

- WT PROTEIN
- mRNA
- ATG
- STOP
- TARGET GENE FOR MSI
Replication slippage, mutator pathway and MSI

<table>
<thead>
<tr>
<th>Gene targets:</th>
<th>Cell death regulators</th>
<th>DNA repair pathways</th>
<th>Cell proliferation pathways</th>
</tr>
</thead>
<tbody>
<tr>
<td>CASP5</td>
<td>BLM</td>
<td>RAD50</td>
<td>TGFβRII</td>
</tr>
<tr>
<td>FAS</td>
<td>BRCA2</td>
<td>IGFR1</td>
<td>PTEN</td>
</tr>
<tr>
<td>BAX</td>
<td>MSH1</td>
<td>AXIN2</td>
<td>TCF4</td>
</tr>
<tr>
<td>APAF1</td>
<td>MSH6</td>
<td>PTEN</td>
<td>RIZ</td>
</tr>
<tr>
<td>BCL10</td>
<td>DNA-PKcs</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ATR</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

10%–15% of sporadic colon tumors have MSI
95% of HNPCC tumors have MSI at multiple loci

Chromosome instability & cancer

- Variation in gross chromosome number (aneuploidy)
- Increased rate of chromosome alterations

Oncogenes

Oncogenes are mutated in ways that cause genes to be constitutively active, or active under conditions when wild type genes (proto-oncogenes) are not. Analogous to a stuck accelerator in a car, the car still moves forward even when the driver removes his foot.
Oncogenes

Table 1 Examples of human oncogenes

<table>
<thead>
<tr>
<th>Oncogene</th>
<th>Originally identified in</th>
<th>Mechanism of activation in human tumours</th>
<th>Location</th>
<th>Associated human cancers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Src</td>
<td>Rous sarcoma virus</td>
<td>Oncoproteins, Cytosolic</td>
<td>Cytosolic</td>
<td>Breast, colon, lung</td>
</tr>
<tr>
<td>Myc</td>
<td>Avian myeloblastosis virus</td>
<td>Oncoproteins, Cytosolic</td>
<td>Cytosolic</td>
<td>Bladder, lymph</td>
</tr>
<tr>
<td>Rras</td>
<td>Rous sarcoma virus</td>
<td>Oncoproteins, Cytosolic</td>
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<td>N-Myr</td>
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<td>Oncoproteins, Cytosolic</td>
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KNUDSON TWO HIT HYPOTHESIS IN FAMILIAL CASES OF RETINOBLASTOMA (1971)

Tumor suppressor genes

- Targeted in opposite way by genetic alterations:
  Mutations reduce the activity of a Tumor Suppressor gene.
- Defined as recessive genes, i.e. they must sustain mutations or deletions in both alleles to contribute to cancer.
- What type of mutations?
  Missense mutations, truncated proteins, deletions, insertions, epigenetic silencing.

Analogous to a non-functional brake in a car. Doesn't stop even when driver steps on the brake.

Inactivation of Rb tumor suppressor gene requires two mutations:
  - an inherited mutation
  - a somatic mutation.
KNUDSON TWO HIT HYPOTHESIS IN SPORADIC CASES OF RETINOBLASTOMA (1971)

Inactivation of Rb tumor suppressor gene requires two somatic mutations.

Tumor suppressor genes

<table>
<thead>
<tr>
<th>Name</th>
<th>Function in normal cells</th>
<th>Associated cancers</th>
</tr>
</thead>
<tbody>
<tr>
<td>p53</td>
<td>Cell cycle regulator</td>
<td>Colon and others</td>
</tr>
<tr>
<td>BRCA1</td>
<td>Cell cycle regulator, genetic</td>
<td>Breast, ovarian, prostate and other</td>
</tr>
<tr>
<td>BRCA2</td>
<td>Integrity and chromatin structure</td>
<td></td>
</tr>
<tr>
<td>PTEN</td>
<td>Tyrosine and lipid phosphate</td>
<td>Prostate, glioblastoma</td>
</tr>
<tr>
<td>APC</td>
<td>Cell adhesion</td>
<td>Colon</td>
</tr>
<tr>
<td>MCC</td>
<td>Inactivated</td>
<td>Colon</td>
</tr>
<tr>
<td>RB1</td>
<td>Cell-cycle regulator</td>
<td>Colon and others</td>
</tr>
<tr>
<td>RB2</td>
<td>Inactivation of RB tumor</td>
<td>Colorectal and glioblastoma cancer</td>
</tr>
<tr>
<td>NF1</td>
<td>Regulation of GTPases</td>
<td>Neurofibromatosis</td>
</tr>
<tr>
<td>NF2</td>
<td>Cell adhesion</td>
<td>Not known</td>
</tr>
<tr>
<td>VHL</td>
<td>Ubiquitin</td>
<td>Renal</td>
</tr>
<tr>
<td>PTEN</td>
<td>Regulation of lipid-signaling</td>
<td>Brain, esophageal</td>
</tr>
<tr>
<td>CTN</td>
<td>Cell cycle regulator</td>
<td>Breast and renal</td>
</tr>
<tr>
<td>TSC2</td>
<td>Cell cycle regulator</td>
<td>Renal and brain</td>
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Summary: Mutations, genetic instability & cancer

Mutations of tumor suppressor genes lead to cancer development. Genetic instability can also occur, leading to further mutations.
Models of Carcinogenesis

1. The single mutation model
Chronic myelogenous leukemia (CML):

95% of CML patients carry the ‘Philadelphia’ chromosome

What is BCR-ABL?

- BCR gene on chromosome 22
- ABL gene on chromosome 9

CML breakpoints
P210 Bcr-Abl
Models of Carcinogenesis

1. The single mutation model
Chronic myelogenous leukemia (CML):
Why is BCR-ABL oncogenic?

What is the evidence of the single mutation driving cancer?
Models of Carcinogenesis

2. The Vogelstein model of colorectal carcinogenesis (1993)

Mutated in 70% of Familial adenomatous polyposis
Deleted in 73% of colon cancers

Six distinct alterations in cell physiology that dictate malignant growth.

Six distinct alterations in cell physiology that dictate malignant growth.
1. Self-sufficiency in growth signals

Growth factor signaling

- diffusible growth factors
- extracellular matrix components
- cell-to-cell adhesion/interaction molecules

Quiescent state

Proliferative state

Normal cell

Many oncogenes override the requirement for growth factors for proliferation

Tumour cell
1. Self-sufficiency in growth signals

Oncogene-mediated molecular strategies for achieving growth factor autonomy:
- alteration of extracellular growth signals (autocrine stimulation).
- alteration of transcellular transducers of those signals.
- alteration of intracellular circuits that translate those signals into action.

GF (hypersensitivity)               ligand-independent growth
Typical examples:
EGFR: breast, brain, stomach
HER2/neu: breast, stomach
Integrins: heterodimeric ECM receptors
αvβ3 enhances tumour growth in melanomas
α2β1 enhances invasiveness in breast cancer
1. Self-sufficiency in growth signals

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1. Self-sufficiency in growth signals

The ras signalling pathway

<table>
<thead>
<tr>
<th>Human tumours exhibiting mutated Ras &amp; Raf</th>
<th>Colon</th>
<th>Pancreatic</th>
<th>Ovarian</th>
<th>Melanoma</th>
<th>Papillary thyroid</th>
<th>ALL, AML</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumours</td>
<td>Ras (45%)</td>
<td>BRaf (12%)</td>
<td>Ras (90%)</td>
<td>Ras (15%)</td>
<td>Ras (60%)</td>
<td>Ras (30%)</td>
</tr>
</tbody>
</table>

2. Insensitivity to anti-growth signals

Cell cycle (cell division cycle): essentially the process of cell replication
- complex, highly conserved process
- regulated by extracellular and intracellular signals

Anti-growth signals operate to maintain cellular quiescence and tissue homeostasis.
Anti-growth signals are delivered by soluble growth inhibitors or immobilised inhibitors for example ECM components.
2. Insensitivity to anti-growth signals: central role for pRb

- Hypo-phosphorylated pRb and the related p107 and p130 sequester E2F transcription factors that drive the expression of cell cycle-regulatory genes – absence of Cdk activity.
- Mitogens induce cyclin D expression that results in active Cdk4/6-cyclin D.
- These complexes phosphorylate and inactivate pRb thus releasing E2F.

2. Insensitivity to anti-growth signals

- The TGFβ paradigm

Normal cells

- Down-regulation of TGFβRs
- Mutations in TGFβR that render them dysfunctional
- Mutations in SMAD4
- Deletion of the p15(INK4B) locus
- Mutations in cdk4 that render them unresponsive to p15(INK4B)
- Loss of pRb function by mutation or binding of viral oncoproteins

Cancer cells

Release from anti-growth signals

Inhibition of proliferation
3. Evading apoptosis

Apoptosis

Two major pathways:
1. Intrinsic (mitochondria)
2. Extrinsic (death receptors)

Both pathways:
1. Branch into many pathways
2. Converge on caspase activation
3. Lead to DNA degradation & cell death

Cancer cells
- Elevated NF-κB activity (e.g. Hodgkin lymphoma)
- Mutated p53 (approx. 50% of all cancers)
- Over-expressed Bcl-2 (Lymphomas and carcinomas)
- Activated PI3-kinase pathway (e.g. ovarian cancer)
- Decoy death receptors (e.g. colorectal and lung cancer)

4. Limitless Replicative Potential

Acquired capabilities:
- Growth signal autonomy
- Insensitivity to anti-growth signals
- Apoptosis

Unlimited proliferation & generation of vast numbers of tumours?

Senescence is a barrier to cancer

Activation of the senescence program limits replicative lifespan

Over-regulation of normal cellular program
4. Limitless Replicative Potential

Replicative lifespan is controlled by telomere shortening.

**Telomeres: the ‘cellular clock’**

- Telomeres shorten every division as a result of the mechanism of DNA replication.
- Function as a cellular clock, telling cells how many replications they can make.
- Cells stop dividing when telomeres get “too” short.
4. Limitless Replicative Potential

Cells that can still divide (loss of p53/pRb) AND have lost their telomeres will develop an unstable genome.

1. Overexpress telomerase = limitless replicative potential
2. Inactivate RB = insensitivity to anti-growth signals
3. Inactivate p53 = evasion of apoptosis
4. Limitless Replicative Potential

Is telomere stabilisation an important step towards tumour development?

- Tumour cells have shorter telomeres compared to normal surrounding tissue.
- Most tumour cells express telomerase (i.e. TERT amplification or oncogene-induced up-regulation).
- Expression of TERT rescues HDF from senescence in vitro.
- Down-regulation of telomerase induces apoptosis of tumour cells in vitro.

However:

- Mice lacking the integral RNA template of telomerase (TR) are MORE sensitive to induced tumourigenesis (telomeres shortening is enhancing the frequency of cancer rather than protecting from it?).

Telomeres have dual effects depending on the cell type and the presence or not of gene mutations.

4. Limitless Replicative Potential

Genetic instability overrides the protective role of senescence

In the absence of mutagenic environment replicative senescence protects against tumorigenesis.

- Cells have built-in checkpoints that prevent oncogenes and tumor suppressors from causing neoplasia.
- The senescence checkpoint is controlled by Telomerase.
- The crisis checkpoint is largely controlled by pRb/p53.
- Once bypassed, the cell is ‘immortal’ and neoplasia can occur.
- Telomeres balance the fate of the cell: replicative senescence vs genetic instability.
5. Sustained angiogenesis.

Angiogenesis: the growth of new blood vessels from the pre-existing vasculature.

Key for tumor growth: does not influence cell proliferation but in the absence of oxygen and nutrients there is high rate of apoptosis.

Major target for cancer therapy: Inhibition of new vessel formation would restrict tumor growth.

Cell types involved:
- endothelial cells
- vascular smooth muscle cells
- bone marrow-derived cells
- Tumor cells

Figure 2: The angiogenic cascade. (a) Angiogenic stimuli; (b) Degradation of the basement membrane and ECM by proteases released from tumor and activated endothelial cells; (c) Migration of ECs towards angiogenic stimuli; (d) Endothelial cell proliferation; (e) Tube formation and stabilization.
5. Sustained angiogenesis.

Angiogenic response:
- positive signals (VEGF, FGF, MMPs)
- negative signals (thrombospondin, β-interferon)

VEGF
Permeability of endothelial layer

EC mitogen
EC chemoattractant

• VEGF+/− mice die in utero due to cardiovascular defects (defects in early blood vessel formation)
• Tumor cells expressing VEGF grow faster and contain many blood vessels.

Figure 2. The angiogenic cascade. A. Angiogenic stimuli: 1. Degradation of the basement membrane and ECM by proteases released from tumor and activated endothelial cells; 2. Migration of EC towards angiogenic stimuli; 3. Endothelial cell proliferation; 4. Tube formation and vessel maturation.

How?
Hypoxia → HIF → Hypoxia Response Element in VEGF promoter

α-VEGF Abs inhibit tumor growth in vivo.

VEGF Induces the expression of SDF-1 and synergises with bFGF for angiogenesis.

Hypoxia induces VEGF.

VEGF+−/− mice die in utero due to cardiovascular defects (defects in early blood vessel formation)
• Tumor cells expressing VEGF grow faster and contain many blood vessels.

Oncogenic ras induces VEGF.

VEGF+−/− mice die in utero due to cardiovascular defects (defects in early blood vessel formation)
• Tumor cells expressing VEGF grow faster and contain many blood vessels.
5. Sustained angiogenesis.

Angiogenic response:
- positive signals (VEGF, FGF, MMPs)
- negative signals (Thrombospondin, i-Interferon)

**Induce the production of proteases by ECs**

**MMPs**
- Produced by tumor cells, Fibroblasts, TAMs & ECs

**Breakdown of the basement membrane**

Bevacizumab Binds and neutralizes VEGF

Anti-VEGF antibody (Bevacizumab)

Endothelial cell

5. Sustained angiogenesis.

Angiogenesis: the growth of new blood vessels

Thrombospondin-1
- Binds CD36 on EC.
- Suppresses angiogenesis.
- Regulated by p53: loss of p53 decreases thrombospondin-1 levels.
5. Sustained angiogenesis.

Conclusions
- Angiogenesis, the growth of new blood vessels, appears to be a midstage event in human cancer.
- Neo-vascularization is a prerequisite to the rapid clonal expansion associated with macroscopic tumors.
- Tumor cells control angiogenesis regulators to their own ends.

6. Tissue invasion and metastasis.

Metastasis: The spread of cancer from a primary site to distant organs and the formation of new tumors.

Is metastasis an important issue?
- 90% of human cancer deaths are caused by metastases.
6. Tissue invasion and metastasis.

**Why do different types of cancer associate with different metastases?**

- Different vascular flow patterns

Colon cancer cells are carried by the closed flow to the liver and then the lung. Some may be transported through the systemic arterial system to bone and other remote organs.


6. Tissue invasion and metastasis.

Which are the pathogenic steps towards metastasis?

- Cell binding to basement membrane via adhesion molecules.
- Loss of cell-to-cell contacts

6. Tissue invasion and metastasis.

- Cell binding to basement membrane via adhesion molecules.
- Loss of cell-to-cell contacts.
- Production of matrix-degrading proteases (e.g., MMPs).

E-cadherin:
- Conveys anti-growth signals channeled via β-catenin/TCF.
- Mutational inactivation in cancer.
- Forced expression of E-cad suppresses invasive tumour phenotype in mice.

MMPs: a family of proteolytic enzymes.
- Proteolysis ECM components.
- Facilitate tumour cell invasion through physical barriers (blood vessel walls, stroma etc.).
- Produced by tumour cells or by conscripted stromal and immune cells.
- Other functions:
  - Cleave/activate growth factors.
  - Process cell adhesion molecules.
  - Facilitate resistance to apoptosis.

6. Tissue invasion and metastasis.

- Cell binding to basement membrane via adhesion molecules.
- Loss of cell-to-cell contacts.
- Production of matrix-degrading proteases.
- Changes in integrin expression to adapt to tissue microenvironments.

The Hallmarks of Cancer - Summary

- Different order in different cancer types.
- Particular genetic lesions may confer several capabilities simultaneously.
- Collaboration of two or more distinct genetic changes to acquire a capability.
- Cancer development critically depends on interactions between cancer cells and their environment.

Evading immunosurveillance: the 7th hallmark of cancer?
Mechanisms of tumor escape from the immune system

Loss of antigen processing machinery
Tumor cell-mediated suppression of DC and T cell function

VLC: vascular leukocyte cells
pDC: plasmacytoid DC
NKT: Natural killer T cells
PD1: Program Death 1
MSC: Myeloid suppressor cells

Cancer: General Etiology and Pathogenesis