Oncogenes and tumour suppressor genes

Cancer mutations disrupt cellular homeostasis

Oncogenes: Gain of function mutations

- Proto-oncogene
- Oncogene
- Oncogene

- Normal protein but quantitative increase due to altered regulation
- Abnormal protein due to mutations causing change in protein function
- Abnormal fusion protein due to gene rearrangements. Change in structure and function

Tumour suppressor genes: loss of function mutations

- Normal cell
- Cancer cell

- active growth promoter
- active growth promoter
- active growth promoter

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**Acronimi**

- Myc  Mielocitomatosi
- Sis  Sarcoma delle scimmie
- Erb  Eritroblastoma
- Ras  Sarcoma del ratto
- Abl  Virus della leucemia murina di Abelson

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**List of oncogenes**

- Growth factor signalling regulates cell proliferation

1. Growth Factor
2. Growth Factor Receptor
3. Intracellular domain

- Signal transducers
- Transcription Factor
- Cell proliferation proteins

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**Effetto del PDGF sulla proliferazione di cellule normali o insensibili al PDGF**

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How cancer cells achieve growth factor autonomy

1. Growth Factor
2. Growth Factor Receptor
3. Signal transducer
4. Transcription Factor
5. Nucleus
6. Cell proliferation protein

Alterations in growth factors E.g. PDGF

- C-sis encodes a growth factor PDGF-β
- V-sis is a virally encoded oncogene
- Cells infected with v-sis, overproduce PDGF-β causing constitutive growth stimulation
- C-sis can also be overexpressed without viral infection
- Occurs in sarcomas and astrocytomas

Alterations in growth factor receptors

Mutations that can either result in

A. Increase in receptor density (overexpression)
B. Ligand-INDEPENDENT signalling (changes in binding site)
C. Receptor switching
Alterations in growth factor receptors

Mutations that can either result in
A. increase in receptor density (overexpression)
B. Ligand-INDEPENDENT signalling (changes in binding site)
C. Receptor switching

Metaplastic breast carcinomas

70–80% of MBCs overexpress the Human Epidermal growth factor Receptor

E.g. Mutations in the Epidermal Growth Factor Receptor (EGFR)
Deletion mutation removes exons 2-7 in the extracellular domain leading to ligand-independent activity

Cancer cells preferentially express extracellular matrix receptors (integrins) that favour growth
Changes in intracellular signalling messengers

Growth Factor Receptor
Extracellular domain
Intracellular domain

Signal transducer

Transcription Factor
Nucleus

Cell proliferation protein

E.g. c-crk (cell cycle related kinase)

Bcr-abl viral oncoprotein activates c-crk

Transcription Factor
Nucleus

Cell proliferation protein
Sequence Changes in Viral Src

To date, 4 Src inhibitors, including Dasatinib, have reached clinical trials in patients with prostate cancer.

 Src family kinases are activated in human cancers and Src-activated pathways contribute to tumor progression.

Changes in intracellular signalling messengers
E.g. Ras (small GTP-binding signalling molecule)

Cancer cells contain various viral forms such as v-Ha Ras, v-Ki ras, N-ras.

Chemical carcinogens results in mutations at residues 12, 13, 59 and 61.
Changes in intracellular signalling messengers

E.g. transcription factors like c-myc

Epstein–Barr virus (EBV) associated viral proteins, EBNA1, LMP-1 and -2A, constitutively activate c-myc oncogene by decreasing ubiquitin-dependent proteolysis of this protein in Burkitt’s

Other causes - Chromosomal rearrangements leading to altered regulation

Burkitts lymphoma
Some patients show translocation of the immunoglobulin gene on chromosome 14 to the c-myc oncogene locus on chromosome 8 t(8:14) c-myc is under regulatory control of IgH resulting in overexpression of the oncogene

Chromosomal rearrangements - fusion gene

Chronic Myelogenous Leukaemia (CML)
Translocation t(9:22)
Abl-bcr fusion gene encodes a constitutively active protein tyrosine kinase, which affects cell cycle, adhesion and apoptosis

GLEEVEC – inhibits activity of tyrosine kinase by competing for the ATP binding site

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Meccanismi di attivazione degli oncogeni cellulari

- Mutazioni (puntiformi, delezioni, inserzioni)
  - Ras
- Amplificazione genica
  - Erb-B2, Myc
- Riarrangiamento dei cromosomi (traslocazione, inversione)
  - Bcl 2, Myc
- Fusione di geni che codificano per proteine chimeriche
  - BCR/Abl

Tumour suppressor genes
loss of function mutations

- TSG mutations mainly recessive

Normal cell  Cancer cell

Anti-growth signals...

- monitor cellular environments and force cells into G0 if needed
- maintain post-mitotic status following differentiation
Some important tumour suppressor proteins

<table>
<thead>
<tr>
<th>Name</th>
<th>Chromosome</th>
<th>Location</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>RB</td>
<td>13</td>
<td>Nucleus</td>
<td>Cell cycle</td>
</tr>
<tr>
<td>p53</td>
<td>17</td>
<td>Nucleus</td>
<td>DNA repair, apoptosis</td>
</tr>
<tr>
<td>tum2</td>
<td>17</td>
<td>Mitochondria</td>
<td>Mutations</td>
</tr>
<tr>
<td>BCL-2</td>
<td>17</td>
<td>Nucleus</td>
<td>DNA repair</td>
</tr>
<tr>
<td>APC</td>
<td>5</td>
<td>Cytoplasm</td>
<td>Cell-cell recognition</td>
</tr>
</tbody>
</table>

p53 mutations and cancer
- ~50% of all cancers show mutations in p53
- 90% mutations in Squamous Cell Carcinoma (SCC)
- 80% point mutations and 20% truncations
- Mutations in p53 cause loss of function
- leads to continued cell division despite DNA damage
- leads to increased mutation rate

TP53 – the gene
- Chromosome 17p13.1
- TP53 contains 11 exons
- No TATA box in promoter region
- Contains consensus binding sites for transcription factors such as NF-kappaB/ c-jun
- TP53 expression is ubiquitous and constitutive

p53 protein domains
- Transactivation Domain (TAD) 1-44, 43-62
- Proline rich 65-97
- DNA binding 102-292
- Tetramer Formation 223-356
- Regulatory Domain 363-393

circles, S/T phosphorylation sites; hexagons, acetylation sites; octagons, sumoylation site.
Initially identified as a tumour specific nuclear antigen of 53kDa
4 monomers form a functional tetramer
When wild-type gene transfected into tumours, it stopped their growth

**p53 – 'guardian' of the genome**

- Cellular stress
- Post-translational modifications
- Cell cycle arrest
- Stimulates DNA repair
- Prepares for apoptosis

**Activation and response of p53**

- p53 accumulates by the dissociation of the p53–Mdm2 interaction due to either
  1. mdm2 sequestering proteins like ARF (alternative reading frame; p14ARF in human).
  2. Post-translational modification

**Oncogenic activation**

**DNA damage**

**Regulation of p53 by mdm2**

Overexpression of mdm2 switches off p53

By binding to the transactivation domain of p53 and blocking its ability to activate transcription

- E.g. de novo glioblastoma multiforme
Exposure to stress induces post-translational modifications

Nature Reviews Cancer 4, 793-805 (October 2004)

p53 – tetramerisation domain

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<th>Proline rich</th>
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<th>Tetramer Formation</th>
<th>Regulatory Domain</th>
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DNA binding 102-292

Regulation of p53

3 phases
1) Activation phase
2) Effector phase
3) Outcome phase

Mutations in tetramerization domains

35/36 children in southern Brazil with adrenocortical carcinoma (ACC) had a R337H mutation within tetramerization domain of p53

Nature Structural Biology 9, 121-121 (2001)
1. Activation phase
Stress signalling pathways increase levels of stabilised p53 with increased specific activity

2. Effector phase
Transactivation domain
DNA binding
Tetramer formation
Transcriptional inhibition

Gene activation: Binds to p53 response element on response genes
Gene inhibition: Binds to transcription factors required by other genes

3. Outcome phase
Cell cycle arrest..

...and apoptosis

Stress-induced p53 results in transcription of p21
Mutations in the DNA binding domain of p53 results in loss of DNA binding affinity
Oncogenes and tumour suppressor genes – Retinoblastoma protein

**Key concepts**
- Cell cycle clock operates through serine/threonine kinases (CDKs) which are activated by their partners, cyclins
- D-type cyclins are key in transmitting extracellular mitogenic signals to the cell cycle machinery
- Many cancer cells inactivate this delicate control (especially the late G1 phase R point)
- Phosphorylation state of pRb controls this checkpoint
  - Hypophosphorylation blocks R passage (Binds E2F)
  - Hyperphosphorylation permits R passage (E2F release)
- Viral oncoproteins mimic hyperphosphorylated pRb
- pRb function lost by
  - excessive mitogenic signals,
  - Rb mutations,
  - binding by viral (E7)

Many cancer cells inactivate the delicate control of R point
Phosphorylation state of pRb controls this checkpoint

Hypophosphorylation blocks R passage (Binds E2F)

Hyperphosphorylation permits R passage (E2F release)

Retinoblastoma

Rare childhood cancer of the eye (retinomas) that develops in children, typically under five years old.

Incidence
- 2% of childhood malignancies

Influencing factors
- 30-40% hereditary
- 60-70% sporadic

Treatment
- Surgery, radiation, chemotherapy

Molecular genetics of Rb

The 2-hit hypothesis

Loss of heterozygosity (LOH)

Retinoblastoma protein (pRb)

- Normally inhibits cell proliferation
- Localised in the nucleus
- Rb gene is 300kb long & mutations in this gene leads to loss of function.
- Tumour suppressor protein of ~110kD
- pRb has >10 phosphorylation sites (affects protein-protein interaction)
- Most mutations involve chromosomal changes in the 3kb coding region and 1/3 are point mutations.
- Loss of heterozygosity at chromosome 13q14.2.
Viral oncproteins (E7) mimic hyperphosphorylated pRb

Viral protein (E7) binds pRb

pRb function lost by

- binding by viral (E7) or cellular (myc) oncoprotein
- excessive mitogenic signals
- Rb mutations
**Key concepts**

- Cell cycle clock operates through serine/threonine kinases (CDKs) which are activated by their partners, cyclins
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**Some thought questions on oncogenes & TSGs**

- Why are most familial cancers associated with mutations in TSGs and very rarely with oncogenes?
- Is the activity of oncoproteins and tumour suppressors a cumulative effect?
- Why do Rb-/- patients get mainly retinal cancer?
- Is cancer a disease not of cell division but of cell differentiation?