Molecular Medicine - Principles of Cancer Genetics - Lectures 1 & 2

Dr. Maureen J. O’Sullivan
Consultant Pediatric Pathologist
Our Lady’s Children’s Hospital and National Children’s Hospital
Cancer

Second commonest cause of death in the Western world. Affects all ages and all ethnicities.

Not just one disease – called after tissue of ‘origin’ or what the cells differentiate towards, many subtypes even within this schema. Many types of Leukemia, many types of Kidney cancer etc.

Mol med TCD 2011 Lecture Prof. MO’Sullivan
Kidney Cancer
Many different types even in the pediatric group!

Mol med TCD 2011 Lecture Prof. MO’Sullivan
MALT Lymphoma – link with Helicobacter
Cancer-Host and Cancer-Environment Interactions

Environmental factors – lifestyle; exposures; infectious agents – viral oncogenesis lecture to come... Prevention: Anti-sunbed; anti-smoking etc.

Host factors including immune system – chronic inflammatory / autoimmune disorders as precursors to cancer – IBD; HLO; GERD; concept of immune surveillance - melanoma

Genetic Predisposition – Familial cancers and cancer predisposition syndromes
Cancer development results from an... Interplay that is complex and multifactorial
Molecular Medicine of Cancer

This course will explain the molecular genetic basis of cancer development and progression
Growth vs. Neoplasia/‘New Growth’

Growth is essential in development

Needs to occur in an organised gradual regulated fashion - a programmed series of events largely genetically predetermined, but environmental factors also important – starvation, neglect, hormonal environment etc...

Results from the organised and programmed accumulation of cells throughout all tissues.

Once growth is complete, there is balanced cell turnover – homeostasis where net proliferation of cells is in balance with programmed cell death.
Side-effects of traditional cytotoxic therapy include mucositis, bone-marrow suppression, alopecia – want to fine-tune therapy to target~ONLY cancer cells.
Molecular Biology of Cancer – the Era of Molecular Therapeutics

Understanding abnormalities that govern the uniquely cancerous behavior in cells facilitates targeted treatment of cancers

Mol med TCD 2011 Lecture Prof. MO’Sullivan
Neoplasm [new growth]

Definition:

“A neoplasm is an abnormal mass of tissue, the growth of which exceeds and is uncoordinated with that of the normal tissue and persists in the same excessive manner after cessation of the stimuli which evoked the change.”

Sir Rupert Willis, 1952.

Neoplasm = Tumor = Swelling; Oncos [Ονχοσ] – ‘oncology’

Mol med TCD 2011 Lecture Prof. MO’Sullivan
Tumorigenesis

Tumor development - How do we get an ‘abnormal mass of tissue’?
Normal cell turnover requires balance between cell proliferation and cell death*. Net positive balance leads to cellular accumulation.

Proliferation a function of the cell cycle;
*Programmed cell death = apoptosis
– details to follow.....

Mol med TCD 2011 Lecture Prof. MO’Sullivan
The Cell Cycle...full lecture to follow

GROWTH INHIBITORS (TGF-β, p53, others)

Stimulate

CDK Inhibitors e.g., *p16 (INK4a)

Inactivate

*Cyclins D/CDK *4,6
Cyclin E/CDK2

G1

S

G2

M

G0

GROWTH FACTORS (EGF, PDGF)

Activate

Mol med TCD 2011 Lecture Prof. MO’Sullivan
Control of Cell Population - Apoptosis

Mol med TCD 2011 Lecture Prof. MO’Sullivan
Apoptosis

60 billion new cells generated daily, need to lose equal number
Development of Neoplasms

Initial changes facilitating cellular accumulation – some growth advantage conferred on the tumor cells over background normal cells.

The process doesn’t necessarily stop at primary mass formation – benign versus malignant tumors.

Mol med TCD 2011 Lecture Prof. MO’Sullivan
Neoplasm = Tumor

Benign tumor = non-lethal of its own inherent biological potential- may still be lethal if unfortunate location produces pressure effects with compression of vital structures.

Malignant tumor has the biological potential to kill the patient – ie inherently possesses the ability to metastasise.

Mol med TCD 2011 Lecture Prof. MO’Sullivan
Spread

Local - direct extension into surrounding tissue - margins of neoplasm – pushing or infiltrative

Distant – spread through body cavity, lymphatics, blood vessels to reach distant sites, including lymph nodes, lungs, liver, bone marrow, brain

Metastasis ['secondaries'] – the hallmark of malignancy
Neoplasms – Benign and Malignant

Benign tumors are often slow-growing and well-encapsulated, compress surrounding tissue rather than invading it.

Malignant tumors often infiltrate and destroy surrounding tissues, may grow more rapidly and spread within the patient = CANCER.
Benign Tumors
Malignant Tumor
Metastatic Disease
Invasion

Local first......... then distant

For tumor to be invasive, it must breach its basement membrane – before that, all changes at most amount to *in-situ* malignancy.
Clonal expansion, growth, diversification, angiogenesis

Metastatic subclone

Adhesion to and invasion of basement membrane

Passage through extracellular matrix

Intravasation

Interaction with host lymphoid cells

Tumor cell embolus

Adhesion to basement membrane

Extravasation

Metastatic deposit

Angiogenesis

Growth

Mol med TCD 2011 Lecture Prof. MO’Sullivan
Tumor Microenvironment

Tumor is not an island – needs to interact with the surrounding tissue including circulating cells of immune system and needs to establish its own blood supply.

Rapid growth $\Rightarrow$ high $O_2$ demand

Tumor Angiogenesis a major focus of research and therapeutic investigation

Mol med TCD 2011 Lecture Prof. MO’Sullivan
Angiogenesis – simplistic schema

Mol med TCD 2011 Lecture Prof. MO’Sullivan
Progression in the context of Malignancy

A whole series of genes is involved in the progression of cancer, including localised growth with neo-angiogenesis, altering tissue proteins to permit invasion locally, entry into the lymphatics and/or blood stream, survival of tumor emboli in the bloodstream in transit and ability of the tumor emboli then to become adherent and develop their own blood supply at the metastatic site and continue to grow there.
Cancer development is a multistep process underpinned by genetic progression, often with microscopic +/- gross manifestations at each step in the process

- see adenoma-carcinoma progression in colorectum

Mol med TCD 2011 Lecture Prof. MO’Sullivan
NORMAL COLON

MUCOSA AT RISK

ADENOMAS

CARCINOMA

Mucosa
Submucosa
Muscularis propria

Germline (inherited) or somatic (acquired) mutations of cancer suppressor genes ("first hit")

Methylation abnormalities Inactivation of normal alleles ("second hit")

Protooncogene mutation

Homzygous loss of additional cancer suppressor genes

Additional mutations Gross chromosomal alterations

APC at 5q21
Mismatch repair genes, e.g., MSH2 at 2p22

APC β-catenin MSH2

K-ras at 12p12

p53 at 17p13 LOH at 18q21

Many genes

Mol med TCD 2011 Lecture Prof. MO’Sullivan
What governs this multi-step process?

Cancer genetics

Mol med TCD 2011 Lecture Prof. MO’Sullivan
DNA makes RNA makes Protein

Changes in the DNA or RNA [direct or indirect] will affect the functional outcome by altering the encoded protein

Quantitative or qualitatively [sequence-structure-function]

Direct change = genetic mutation = DNA sequence change

Indirect change epigenetic dysregulation –DNA or RNA = sequence UNchanged
Basically the genes mutated in cancer are considered either oncogenes or tumor suppressor genes.
Oncogene

Definition

“Oncogenes are mutated forms of proto-oncogenes that cause neoplastic transformation by interfering with normal cell growth or differentiation often by disrupting control of the cell cycle.”

~70 known proto-oncogenes

Mol med TCD 2011 Lecture Prof. MO’Sullivan
Tumor Suppressor Genes

“Genes, which by sustaining loss of function mutation, facilitate the development of cancer.”

~20 known Tumor suppressor genes
How are oncogenes ‘switched on’ or tumor suppressor genes ‘switched off’?

To answer these questions, we need to revisit the Genome!
DNA

Genetic Blueprint – this is your germline constitution, which you can pass on to the next generation

Genetic Code – made up of 4 bases = same in all cells [exception is mosaicism] – therefore a germline mutation will be in the DNA in ALL cells

DNA replicated ~ faithfully with each round of mitosis.

Need to guard the integrity of the genome which is constantly under assault: > 10,000 mutations per day! - and with each cycle of DNA replication incur risk of mutation being perpetuated.

DNA repair mechanisms of various types exist... these too may be mutated – see later........

Mol med TCD 2011 Lecture Prof. MO’Sullivan
How is DNA arranged?

Genetic material is housed mainly in the nucleus in the form of chromosomes
[also mitochondrial DNA = of maternal origin]
Chromosomes are identifiable in laboratory context permitting assessment of gross genetic abnormalities – numerical/structural.
G-banded Metaphase chromosomes - karyotype
Chromosome

Chromatin fibre comprises nucleosomes coiled together.

Nucleosome = central 8 histones with DNA wrapped around them. Nucleosomes arranged into ‘beads on a string’.

Tightly wrapped chromatin = heterochromatin – usually genes within heterochromatin NOT transcribed. Loosely wrapped = euchromatin from which genes may be transcribed.
Hierarchy of chromatin structure

DNA and histone protein components

30nm fibre

Nucleosome unit

11nm (beads-on-a-string) fibre

Higher-order chromatin architecture

Mol med TCD 2011 Lecture Prof. MO’Sullivan
Chromatin remodelling – Epigenetic Alteration of the Genome

Epigenetic modification can be of DNA itself or of chromatin - Histone modification: e.g. acetylation of lysine residues leads to loosening of chromatin as negative charge of histones now reduces affinity of histones for $\text{PO}_4^-$ DNA and so can facilitate gene transcription. Known activators of gene transcription have HAT [histone acetyltransferase] activity.

Corollary is that histone deacetylation can repress gene transcription – HDACi in use in cancer treatment.
### Modification States of Histone Tails

<table>
<thead>
<tr>
<th>N-terminal Tail</th>
<th>Modification State</th>
<th>&quot;Meaning&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>unmodified</td>
<td>gene silencing?</td>
</tr>
<tr>
<td></td>
<td>acetylated</td>
<td>gene expression</td>
</tr>
<tr>
<td></td>
<td>acetylated</td>
<td>histone deposition</td>
</tr>
<tr>
<td></td>
<td>methylated</td>
<td>gene silencing/heterochromatin</td>
</tr>
<tr>
<td></td>
<td>phosphorylated</td>
<td>mitosis/meiosis</td>
</tr>
<tr>
<td></td>
<td>phosphorylated/acetylated</td>
<td>gene expression</td>
</tr>
<tr>
<td></td>
<td>higher-order combinations</td>
<td>?</td>
</tr>
<tr>
<td></td>
<td>unmodified</td>
<td>gene silencing?</td>
</tr>
<tr>
<td></td>
<td>acetylated</td>
<td>histone deposition</td>
</tr>
<tr>
<td></td>
<td>acetylated</td>
<td>gene expression</td>
</tr>
</tbody>
</table>

**Diagram:**

- **H2B:**
  - N-terminal tail with modifications at positions 5, 12, 15, and 20.
- **H3:**
  - N-terminal tail with modifications at positions 9, 10, 14, 18, and 23.
  - Distinct modifications (Ac, Me, P) at different positions.
- **H4:**
  - N-terminal tail with modifications at positions S1, K5, K8, and K16.
  - Distinct modifications (Ac, Me, P) at different positions.

**Legend:**
- **Ac:** Acetylation
- **Me:** Methylation
- **P:** Phosphorylation

**Note:**
- "histone-fold domain" indicated in the diagram.
Epigenetics: **DNA** methylation

Usually occurs at either CpG islands [in or near promoters esp.] or CG-rich sequences in repeat regions

Methylation usually ⇒ **Silencing** of the gene

With EPIGENETIC modification, the DNA sequence is unchanged!

Clinical use of Azacytidine
Genetic Mutation = Change of DNA sequence

Nucleotide = Base plus sugar plus phosphate backbone. Phosphodiester bonds hold sugar residues from neighboring bases together.

4 Bases – GCAT; G complements C; A complements T

Orientation 5’ and 3’ depending on where phosphate binds
DNA makes RNA [makes protein] 
What’s the impact of a change in DNA sequence?

Not ALL DNA is transcribed and what’s transcribed is not transcribed continuously. [Developmental- and tissue-specific regulation and response to ‘environmental’ pressures also.] Therefore mutation may effectively be ‘silent’ ...

Mol med TCD 2011 Lecture Prof. MO’Sullivan
DNA Organisation

Not all DNA transcribed: DNA made up of Genes and intergenic regions ['junk’ DNA]

Gene = unit encoding a protein, the functional end-product
<5% DNA ever transcribed into functional end-product

Genes organised into regulatory regions, coding and non-coding regions – exons and introns.
RNA splicing - protein code [open reading frame] comes from exonic sequence only read in triplets
Effect of DNA mutation

Historically, we thought that mutations would only matter if they were in exonic DNA which went on to be part of the protein....NOT SO!!
The Human Genome Project - UCSC Genome browser
DNA transcribed into mRNA

RNA complementary to DNA sequence with U instead of T; G-C and U-A.
RNA polymerase II and complex formation
Start site, stop site = basic RNA. Then splicing, post-transcriptional modification
Translation into Protein – not full mRNA necessarily becomes protein – untranslated regions **where miRNAs often bind**.
Effect of mutation variable

Not all DNA makes RNA and not all RNA becomes protein. While the proteins are the currency of cellular activity, cannot deduce normal function from correct exonic DNA sequence alone....

Modifications/mutations in DNA outside of exonic regions and indeed in chromatin can have profound impact on resultant protein
Effect of Mutation - The Genetic Code

The Genetic Code

Mol med TCD 2011 Lecture Prof. MO’Sullivan
RNA encodes protein

Proteins = the functional end of cell biology

Translation - Start codon, stop codon; post-translational modification

Hormone, Growth factor, Receptor, Signalling protein, structural protein, enzyme etc.
Protein – the functional currency of cell activity – modes of signalling in cells
Signalling cascade within the cell – Signal from the extracellular compartment through receptor binding is transduced via a series of proteins – [many = enzymes] - to the nucleus, resulting in alteration of gene transcription.
Not *ALL* RNA encodes protein

- miRNA
- shRNA
- snRNA
- snoRNA
miRNAs – discovered in 1993 in nematode C. elegans

Very short RNA species
Total number currently known >800 in humans
19-25 nucleotides long
Each miRNA has multiple targets and in all miRNAs thought to regulate ~30% protein-coding genes
Important in development and differentiation; roles in cell cycle and apoptosis regulation
Can be mutated just like regular genes by amplification, deletion, epigenetic change

Mol med TCD 2011 Lecture Prof. MO’Sullivan
miRNA Synthesis

Mol med TCD 2011 Lecture Prof. MO’Sullivan
miRNAs

Can exert effect through inhibiting protein translation or through mRNA degradation.

Often [>50%] located in regions of genomic instability and specific diagnostic and prognostic miRNA profiles evolving for various cancer types.

Oncogenic “Oncomirs” or TSG roles depending on targets – miRNA may act as TSG in one context and oncogene in another. Those that act as TSGs may be located at various genomic loci all giving rise to identical mature miRNA.
miRNAs Role in Oncogenesis

Let-7 can inhibit expression of Ras [common oncogene]
miR-34a overexpression inhibits many pro-proliferative genes and also bcl-2 and induces apoptosis. miR-34a locus frequently deleted in cancer

miR-372 and -373 inhibit LATS2 regulation of CDK2 and so permit activation of cell cycle
May have miRNA cluster at locus that is e.g. amplified and overexpressed in cancer - miR17-92
miRNA in cancer therapy

Antagomirs under investigation = ss antisense / complementary miRNA that bind mature miRNAs
How are oncogenes ‘switched on’ or tumor suppressor genes ‘switched off’?
TSG

Inactivated – HOW? – mechanisms =
Deletion
Point Mutation – remember the genetic code!

Epigenetic Means:
DNA Methylation
Histone modification – e.g. deacetylation
miRNA
APC Mutation [loss of function]
DNA sequencing to detect point mutation

Mol med TCD 2011 Lecture Prof. MO’Sullivan
Oncogene
Activated – HOW? – mechanisms include
Amplification [of DNA]
Activating mutation
Promoter exchange or Novel Fusion oncogene from translocation

Epigenetically – histone acetylation/ altered methylation of DNA
miRNA
Amplification of N-Myc gene
Fluorescent In Situ Hybridisation N-Myc
Neuroblastoma
CXR with pleural effusion
IHC/CISH for HER2 in pleural fluid cell block from a woman with a past history of breast cancer
Chromosomal translocation
Burkitt’s Lymphoma
Cancer is a step-wise progressive disease -

First hit mutation may be hereditary ‘germline’ mutation [can be de novo in the individual involved] occurs in a minority of cancers; or more commonly, an acquired ‘somatic’ mutation. Hereditary more commonly are TSG mutations. Just one mutation can get ball rolling, but not enough for full cancer development.

**NOTE:**
Failure to repair ‘routine’ DNA damage can also be an inherited state ⇒ RISK++++

Mol med TCD 2011 Lecture Prof. MO’Sullivan
Summary of Lecture
Environment-Host-Cancer Interactions
Neoplasia the result of growth dysregulation
– cell cycle and apoptosis
Structure of the genome and mutations
Mutations in DNA vs. Epigenetic alterations –
Chromatin, DNA methylation, miRNAs
Proteins = the key units involved in cellular functions – importance of the genetic code
Oncogenes and TSGs
DNA repair - more to come on these topics
Acknowledgements – Figures taken from:

• Robbins & Cotran “Pathologic Basis of Disease”, Elsevier Press
• Pfeifer JD “Molecular Genetic Testing in Surgical Pathology”, Lippincott Williams and Wilkins
• Cotter TG. Nature reviews 2009; 9:501-7
• Shchors and Evans. Cancer Res. 2007; 67: 7059-1