**Oncogenes and Tumour Suppressor Genes**

by Professor Nigel Gooderham

The cancer cell phenotype includes disregard of signals to stop proliferating; disregard of signals to differentiate; capacity for sustained proliferation; evasion of apoptosis; ability to invade and ability to promote angiogenesis.

The liver is a good example for proliferation. It is one of the largest organs in the body, but most of the cells are quiescent and are not proliferating because they are getting signals from surrounding cells not to proliferate.

The bone marrow is a good example for differentiation. Cancerous blood cells will proliferate, but will remain immature.

Cancer is an inappropriate growth problem due to the capacity for sustained proliferation. The body maintains a non-cancerous state by apoptosis programming. DNA damage causes apoptosis so that the damage is not passed on to daughter cells. Cancer cells evade this. Cancer cells also have the ability to invade - metastasis. They also have the ability to promote angiogenesis so that they can be supplied with nutritional needs.

**The Cell Cycle**

This is the key to life, death and cancer. For example, normally a liver hepatocyte cell is in the G0 stage. It is not proliferating, but is involved in glucose metabolism, lipid biochemistry, etc.

If there is a trigger to enter the cell cycle, the cell goes into the G1 phase. In this phase, the cell gets itself ready for proliferation, by making sure the biochemistry of the cell is ready. There is a checkpoint here, where there is a check for DNA damage and a check to see if the cell is ready for proliferation. If the genome is damaged, the cell will often arrest in the G1 phase.

In the S phase, the entire genome is duplicated. There is another checkpoint here to check that everything is okay. In the G2 phase, the cell is still getting itself ready to divide. There is yet another checkpoint here to check that the cell is ready to produce a daughter cell. The cell then enters mitosis. This is an incredibly well co-ordinated process, with proteins (cyclins, cycle dependent kinases, cycle dependent kinase inhibitors) being synthesised to push it from one stage to another, and other proteins produced to prevent this. These proteins are accumulated or destroyed, and by being so, they allow the progression of the cell through this cycle. It is when this goes wrong, that cancer develops.

**Critical Gene Targets**

Proto-oncogenes code for essential proteins involved in maintenance of cell growth, division and differentiation. These are proteins that push the cell through the cell cycle.

Mutation converts a proto-oncogene to an oncogene, whose protein product no longer responds to control influences. Oncogenes can be aberrantly expressed, over-expressed or aberrantly active (e.g. MYC, RAS, ERB, SIS).

Proto-oncogenes can be converted to an oncogene by a single base mutation.
A **point mutation** is where a base is changed/removed/added. The consequence of this can be activation of a proto-oncogene into an oncogene, with the result of the gene being aberrantly active. **Gene amplification** is where we get multiple copies of the gene in the genome, which is a problem because the protein is going to be produced (e.g.) 3 times as much = overactivity. This problem of multiple copies can occur during replication. In **chromosomal translocation**, there is a swap-over of genetic material to produce **chimaeric genes**. If there is a piece of DNA that shouldn’t be there next to the proto-oncogene, then this can cause activation. This situation is seen in Burkitt’s lymphoma, where there is a strong enhancer right next to the gene to enhance normal protein levels. **Insertional mutagenesis** is where one lump of chromosome is inserted right next to another lump of chromosome. This is often virally delivered, e.g. Philadelphia chromosome, where there is overproduction of the protein or hyperactivity.

**Proteins** involved in signal transduction are potential critical gene targets (proto-oncogenes). In the diagram we see a plasma membrane with a number of receptor type proteins. There are exogenous molecules which can get through the plasma membrane to get to intracellular receptors. The consequence of this is usually **transcriptional events** and **proliferation** (a signalling cascade within the cell). When a proto-oncogene is converted to an oncogene, these signalling processes go wrong. It is the same with a tyrosine kinase receptor and a G-protein coupled receptor, as these both lead to cascades causing proliferation.

**RAS** is a protein that acts at the **G-protein** coupled receptor. Mutant RAS has aberrant activity. Under normal circumstances, it is part of a signalling cascade to instruct the cell to grow. If it binds GTP, it becomes active. It is used by RAS in a phosphorylation cascade. RAS phosphorylates RAF to make it active, and RAF then signals downstream to the next protein in the sequence, instructing the cell to proliferate. Once this activity is passed on, the GTP is hydrolysed to GDP bound to RAS. This now makes RAS inactive, it is a very effective off switch. The GDP is released, and the RAS is ready to bind another GTP if it receives the correct signal.

A **mutated RAS** gene (which can be achieved through a single base damage) produces an **oncogenic protein**. It passes the signal on and activates RAF etc, but unfortunately **can no longer hydrolyse the GTP**. So now the protein is **permanently switched on** and signals downstream to cause proliferation.

**RAS** is involved in the **Mitogen-Activated Protein Kinase (MAPK) cascades**. RAS genes are highly conserved, and present in a huge range of species. Disruption of the genes is likely to push the cell towards cancer. RAS proteins are membrane bound **GTPases**, central to the stimulation of cell proliferation, especially in response to growth factors.

**Oncogenes and Human Tumours**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
<th>Mechanism of activation</th>
<th>Location</th>
<th>Associated human cancers</th>
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<tbody>
<tr>
<td>SRC</td>
<td>Tyrosine kinase</td>
<td>Overexpression/ C-terminal deletion</td>
<td>Cytoplasmic</td>
<td>Breast, colon, lung</td>
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<td>MYC</td>
<td>Transcription factor</td>
<td>Translocation</td>
<td>Nuclear</td>
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<td>JUN</td>
<td>Transcription factor</td>
<td>Overexpression/deletion</td>
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<td>Ha-RAS</td>
<td>G protein</td>
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<tr>
<td>Ki-RAS</td>
<td>G protein</td>
<td>Point mutation</td>
<td>Cytoplasmic</td>
<td>Colon, lung</td>
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Critical Gene Targets

Tumour suppressor genes are typically proteins whose function is to regulate cellular proliferation and maintain cell integrity, so they act like a brake and slow the system down, e.g. retinoblastoma protein. Each cell has two copies of each tumour suppressor gene. Unlike proto-oncogenes, mutation or deletion of one gene copy is usually insufficient to promote cancer. Mutation or loss of both copies means loss of control.

The discovery of tumour suppressor genes was by familial studies. There is inherited cancer susceptibility, as we often see family histories of related cancers. The onset of the problem is usually quite early, because you are born with defective genes as opposed to accumulating mutations through life. There are usually bilateral tumours in paired organs (e.g. kidneys), and synchronous or successive tumours. There are also often tumours in different organ systems in the same individual. This is all because you have inherited the defective gene through the germine.

Retinoblastoma is a malignant cancer of developing retinal cells. Sporadic disease usually involves one eye. Hereditary cases can be unilateral or bilateral and multifocal. It is due to the mutation of the RB1 tumour suppressor gene on chromosome 13q14. RB1 encodes a nuclear protein that is involved in the regulation of the cell cycle.

There are various functional classes of tumour suppressor genes. They regulate cell proliferation, maintain cellular integrity and regulate cell growth. These are all opposite functions to proto-oncogenes. They regulate cell cycle, can act as nuclear transcription factors, DNA repair proteins, cell adhesion molecules and cell death regulators. They suppress the neoplastic phenotype.

p53 is the “conductor of the orchestra”. It regulates all the activities of various pathways in the cell. It is a cell cycle regulator, located in the nucleus. It is associated in many human cancers, as almost 50% of all tumours have damage on p53. BRCA1 is a nuclear cell cycle regulator, associated with breast, ovarian and prostate cancer. PTEN is a tyrosine and lipid phosphatase. It is located in the cytoplasm, and is associated with prostate cancer and glioblastoma. APC is a cytoplasmic gene involved in cell signalling, and is associated with colon cancer. p16 is another nuclear cell cycle regulator associated with colon cancer. MLH1 is a nuclear mismatch repair gene, associated with colon and gastric cancer.

Although p53 is a tumour suppressor gene, mutants of p53 act in a dominant manner and mutation of a single copy is sufficient to get dysregulation of activity.

p53 is kept in check by a protein called MDM2, which binds to p53 and inhibits its activity. p53 is a transcription factor, and one of the proteins it codes for is MDM2. So if there is high p53, it up-regulates MDM2 to limit activity.

p53 is activated by many things, as shown in the diagram on the right. p53 up-regulates many target genes that are also shown in the diagram. If damage to the chromosome is large, then it can also bring about the apoptosis pathway.

p53 is a bit of an exception to tumour suppressor genes, because you only need to damage one copy of the gene in order to produce a problem (i.e. dysregulation of the activities shown above).

The APC tumour suppressor gene is associated with familial adenomatous polyposis coli. It is due to a deletion in 5q21 resulting in loss of the APC gene. The gene is involved in cell adhesion and signalling. Sufferers develop multiple benign adenomatous polyps of the colon. These are not cancerous, but they are highly proliferating and readily accept further genetic damage, which propels the cells towards cancer. There is a 90% risk of developing colorectal carcinoma.
The tumour suppressor gene APC participates in the WNT signalling pathway. This pathway signals using β-catenin, which is a protein which influences transcriptional up-regulation. **APC protein helps control the activity of β-catenin and thereby prevents uncontrolled growth.** Mutation of APC is a frequent event in colon cancer.

The Route to Cancer
For example, colorectal cancer. The normal epithelium will carry out normal functions. If there is mutation of the APC gene (damage or inherited), then we have a hyperproliferative epithelium, and polyps form in the colon. If there is further damage (e.g. DNA hypomethylation or damage to RAS) this is much more aggressive, and you can get an adenoma, which grows out of control, but is benign. Then if there is p53 mutated, everything breaks down and the cell becomes much more nasty - a carcinoma, completely out of control. The next stage is metastasis, and the cells leave the colon and usually go to the liver.

<table>
<thead>
<tr>
<th>Oncogene</th>
<th>Tumour suppressor gene</th>
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<tbody>
<tr>
<td>Gene active in tumour</td>
<td>Gene inactive in tumour</td>
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<tr>
<td>Specific translocations/point mutations</td>
<td>Deletions or mutations</td>
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<tr>
<td>Mutations rarely hereditary</td>
<td>Mutations can be inherited</td>
</tr>
<tr>
<td>Dominant at cell level</td>
<td>Recessive at cell level</td>
</tr>
<tr>
<td>Broad tissue specificity</td>
<td>Considerable tumour specificity</td>
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<tr>
<td>Leukaemia and lymphoma</td>
<td>Solid tumours</td>
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**Learning Objectives for this lecture:**
- Define proto-oncogene, oncogene and tumour suppressor gene.
- Explain how a proto-oncogene can be activated to an oncogene.
- Explain how activating an oncogene can disrupt tightly controlled pathways in the cell.
- Describe how rare heritable cancers have led to an understanding of tumour suppressor genes.
- Summarise the role of the tumour suppressor gene p53 in cellular decision making.
- Describe the way in which successive gene mutations are thought to lead to clinical cancer.
**DNA Damage and Repair**

by Professor Nigel Gooderham

**Damaged genes** can be inherited, but the majority of damaged genes are caused by **chemicals** and **radiation**. Cancer is a multitude of diseases with a multitude of causes.

There are many **chemicals** that can damage DNA, which may be **dietary, lifestyle related, environmental, occupational, medical or endogenous**. Dietary exposure is the primary exposure to chemicals. When you cook a piece of meat, you generate many different chemicals that can damage DNA. We do, however, have fantastic mechanisms within the body to prevent this damage, and things like x-ray scans can cause iatrogenic cancer. In terms of lifestyle, smoking and alcohol can damage DNA. Even medical drugs can damage DNA. Exhaust fumes, working in factories and industry, etc can all damage DNA.

**Radiation** is also dangerous, and this includes ionising radiation, solar radiation and cosmic radiation. Solar radiation is particularly relevant to places like Australia, where skin cancer is prevalent. Cosmic radiation is a real problem, and the higher up in the atmosphere you get the higher the risks.

The problem is that DNA damage can lead to **mutation**, and mutation may lead to **cancer**.

The types of damage that can occur include **DNA adducts** and **alkylation**; **base hydroxylations** and **abasic sites formed**; **base dimers** and **chemical cross-links**; and **double or single strand breaks**.

**Metabolism**

Mammalian metabolism consists of Phase I and Phase II. In **Phase I metabolism**, a **functional group** is added to the xenobiotic. This is done by oxidations, reductions or hydrolysis. It doesn’t change the polarity. These reactions are primarily catalysed by the **cytochrome P450** enzymes, of which there are up to 100 different ones. They have low substrate specificity, and are quite inefficient, but they can metabolise a massive range of chemical structures. In Phase II metabolism, there is conjugation of the Phase I functional groups (adding something on). This includes sulphation, glucuronidation, acetylation, methylation, amino acid and glutathione conjugation. This generates polar (water soluble) metabolites that can be excreted.

**Polycyclic aromatic hydrocarbons** are common environmental pollutants. They are formed from the combustion of fossil fuels, or from the combustion of tobacco. The P450 enzymes in the lung can detoxify these hydrocarbons, but this can go wrong.

Benz(a)pyrene is a common substrate for oxidation by P450. The product formed is a three membered ring called an **epoxide**. This epoxide is highly reactive, and very electrophilic. Electrons are found in proteins, but also in DNA. Epoxide will look wherever it can for electrons to make it more neutral. There is an enzyme called **epoxide hydrolase** which detoxifies the reactive epoxide chemical group by splitting a bond and adding water. This generates a dihydroxy-polycyclic aromatic hydrocarbon. This is a detoxification product, which we can get rid of. However, this product can be a substrate to P450 again. P450 oxidises it to form another epoxide by adding an oxygen across a C=C double bond. **We hope that epoxide hydrolase is able to detoxify all the epoxides, but if P450 generates them faster than epoxide hydrolase can get rid of them, you get electrophilic DNA adducts.** These can damage DNA.

**Aflatoxin** is a product of the *Aspergillus flavus* mould that grows on cereal crops. It is common on poorly stored grains and peanuts. The mould likes hot and humid atmospheres and generates a whole family of toxins. **Aflatoxin B1** is a potent human **liver carcinogen**, especially prevalent in Africa and the Far East, because it is not economically viable for these populations to control food that reaches human consumption. The C=C double bond at the end of the Aflatoxin molecule is a substrate for **P450**, which can again generate an **epoxide**. The epoxide this time, however, **is not a good substrate for epoxide hydrolase**. It attacks the electrons present in the nitrogen of **guanine**. The guanine base is then mutated and damaged. This happens primarily in the liver, which is where most P450 is present.

**2-naphthylamine** used to be used in dyes. Both **Benzidine** and 2-naphthylamine are now banned, because they are directly related to **bladder carcinogenesis**. This was discovered in 1895 by Rehn in a German dye industry. Again they were subject to P450, generating an N-hydroxy derivative. This was detoxified by
glucuronyl transferase in the liver and transported to the bladder. However, the acidic pH of the urine caused the glucuronide group to split off, making it chemically unstable. This then attacked the DNA of bladder cells to cause cancer.

Radiation
Solar radiation is damaging to DNA. For example, there may be pyrimidine (or thymine) dimers, where two bases become cross linked because of the reactivity generated by this radiation. The consequence is skin cancer. Ionising radiation generates free radicals in cells, including oxygen free radicals (super oxide radical and hydroxyl radical). These species possess unpaired electrons, and so they are highly electrophilic and therefore seek out electron rich DNA.

Oxygen free radical attack on DNA may cause double and single stranded breaks. There may also be apurinic and apyrimidinic sites being generated, which is where entire bases are lost. There may also be base modifications like ring-opening of guanine and adenine, thymine and cytosine glycols, or mutagenic 8-hydroxyguanine and 8-hydroxyadenine.

The Role of p53
p53 is the protein that is charged with the responsibility of sorting this kind of damage out. p53 transcriptionally up-regulates MDM2, which switches off p53. p53 is activated by oxidative stress, hypoxia, mitotic apparatus dysfunction, oncogene activation, etc. Once p53 senses these problems, it goes into action. It can up-regulate many target genes to help protect cells against damage: anti-oxidant defence against free radicals, DNA repair genes, growth arresting if DNA damage is quite bad, or even apoptosis programming.

Repair of DNA Damage
The mechanisms in our bodies for DNA damage repair are amazing. The first mechanism is direct reversal of DNA damage, as there is an enzyme called photolyase that splits the cyclobutane pyrimidine dimers caused by solar radiation open again. There are also methyltransferases and alkyltransferases that remove alkyl groups from bases.

Another mechanism is base excision repair, mainly for apurinic or apyrimidinic damage. Here the bases are lost for whatever reason, so there is a gap in the DNA strand. Enzymes such as DNA glycosylases and endonucleases can deal with these situations, and a repair polymerase is involved in filling in the gaps. DNA ligase completes the repair.

Another mechanism is nucleotide excision repair, mainly for bulky DNA adducts. Xeroderma pigmentosum proteins assemble at the site of damage, and a stretch of nucleotides either side of the damage are excised. Repair polymerases fill the gap and DNA ligase completes the repair.

Another mechanism is repair that occurs during or post replication. In S phase, the cell constantly checks the fidelity of the DNA it is producing. If problems are found, these are repaired if possible. The genome is then checked again. There are a number of opportunities for mismatch repair (base pair problems), and recombinational repair (breaks in DNA).

Our cells are constantly under attack. Depurination reactions are happening about 1000 times per cell per hour. This is how much our cells are being attacked. Luckily, our ability to repair these attacks is 10 times greater! Depyrimidination reactions, single-strand breaks and alkylation and free radical base oxidations are all also occurring, but our repair rate is fantastic.

However, the greater the persistence of damage, then the greater the change of a mutagenic event. In the normal situation it would appear that human cells have plenty of spare capacity to deal with both endogenous and exogenous damage but errors creep in especially with increasing age.
The Fate of Carcinogen DNA-Damage

Testing for DNA damage
First of all, you look for chemical structural alerts early on to predict metabolism problems. After this, in vitro bacterial gene mutation assays can be used to pick up mutations, and a classic one used is the Ames test with Salmonella typhimurium. We can also support this with an in vitro mammalian cell mutation assay, culturing mammalian cells in the presence of the chemical to see chromosomal damage, TK mutations, and micronucleus assays. We can also support this with in vivo mammalian assays where we do the same tests in animals e.g. bone marrow micronucleus, which is a good pluripotent stem cell population = leukaemia and lymphoma. Finally, we can do investigative in vivo mammalian assays, the gold standard of looking for chemical carcinogens is the rodent bioassay (feeding the rodent the chemical for its lifetime and seeing if it gets tumours).

The bacterial Ames test for mutagenicity of chemicals is very effective. The chemical to be tested is incubated with the bacteria and a mammalian enzyme preparation (e.g. with P450) is added. The bacteria do not synthesise histidine (e.g. Salmonella strain), so any non-mutated bacteria will die, whereas mutated bacteria will have been mutated to be able to make histidine. So we have effectively selected mutant bacteria and so you can suspect that it can cause cancer.

Another test detects DNA damage in mammalian cells (chromosomal aberrations). The cells are treated with the chemical in the presence of a liver enzyme preparation (S9), and you look for the generation of small pieces of chromosome in the nucleus. Fragments of DNA are an indication of the ability of the chemical to cause problems.

An in vitro micronucleus assay is where cells are treated with the chemical and allowed to divide. Cytokinesis is blocked using cytochalasin-B, and binucleate cells are assessed for the presence of micronuclei. You can stain the kinetochore proteins to determine if the chemical treatment caused clastogenicity (chromosomal breakage) or aneuploidy (chromosomal loss).

Learning Objectives for this lecture:
- Describe how DNA can be damaged by radiation or chemicals (carcinogens) and the role metabolism can play in these reactions.
- Outline in general terms the role of p53 in the detection of, and response to, DNA damage.
- Summarise the natural repair mechanisms for damaged DNA.
- Explain how unrepaired or misrepaired DNA damage can become “fixed” as a mutation.
- Summarise how the potential of a chemical/agent to damage DNA can be assessed.
The Cell Cycle and its Regulation

by Dr. Vania Braga

Different cells divide at different rates, and there are various factors that influence this. Embryonic cells divide faster than adult cells, simple cells divide faster than more complex cells, cells with less necessity to grow (e.g. hepatocytes) divide less, and well differentiated cells like neurons and cardiac myocytes never divide. Tumour cells divide uncontrollably.

Premature or aberrant mitosis can result in cell death. In addition to mutations in oncogenes and tumour suppressor genes, most solid tumours are aneuploid (abnormal chromosome number and content). Various cancer cell lines show chromosome instability (lose and gain whole chromosomes during cell division). These problems are caused by perturbation of protein levels of cell cycle regulators, and this is found in different tumours. Tumour cells lose contact inhibition of growth, whereby normally cells do not grow over on top of each other. Attacking the machinery that regulates chromosome segregation is one of the most successful anti-cancer strategies in clinical use.

The cell cycle is an orderly sequence of events in which a cell duplicates its contents and divides in two. It is a regulated progression. Mitosis consists of nuclear division and then cell division (cytokinesis). Mitosis very fast, and is the most vulnerable period of the cell cycle, as cells are more easily killed (irradiation, heat shock, chemicals), DNA damage cannot be repaired, gene transcription is silenced and metabolism is altered.

Interphase is the longest phase, and this involves the duplication of DNA and organelles and protein synthesis. During G0 the cell cycle machinery is dismantled. G1 is a decision point. S phase involves the synthesis of DNA and proteins. G2 is another decision point.

S phase involves DNA replication. As for protein synthesis, the initiation of translocation and elongation is increased, and the capacity for protein synthesis is also increased. There is also the replication of organelles (centrosomes, mitochondria, Golgi, etc). In the case of mitochondria, this needs to co-ordinate with the replication of mitochondrial DNA.

The centrosome consists of two centrioles (barrels of nine triplet microtubules). It functions as a microtubule organising centre (MTOC) and mitotic spindle. They are always at 90° to each other. The centrosome regulates the choreography of the chromosomes inside the cells.

To duplicate the centrosome, the daughter centriole separates and the two centrioles duplicate. Around the new centrosomes there is a cloud of proteins, with nucleated sites for microtubules. Once everything is duplicated, mitosis begins.
During **prophase**, there is the **condensation of chromatin**. The condensed chromosomes each consist of two sister **chromatids**, each with a **kinetochore**. The replicated chromosomes condense. The duplicated centrosomes **migrate** to opposite sides of the nucleus and organise the assembly of spindle microtubules. The mitotic spindle forms the outside nucleus between the two centrosomes.

In **spindle formation**, radial microtubule arrays form around each centrosome, the radial arrays meet and polar microtubules form. Microtubules are in a dynamic state.

During **metaphase**, the chromosomes are **aligned** at the equator of the spindle. This phase is divided firstly into early prometaphase and late prometaphase. In early **prometaphase**, there is breakdown of the nuclear membrane and spindle formation is largely complete. There is then the attachment of chromosomes to the spindle via **kinetochores** (centromere region of chromosome). In late prometaphase, the microtubule from the opposite pole is captured by the sister kinetochore. Chromosomes attached to each pole congress to the middle. The chromosomes slides rapidly towards the centre along the microtubules.

During **anaphase**, the paired **chromatids separate** to form two daughter chromosomes. Cohesin holds sister chromatids together. During anaphase A, there is the **breakdown of cohesin** and **microtubules get shorter**. Daughter chromosomes are pulled towards the opposite spindle poles. During anaphase B, daughter chromosomes **migrate** towards the poles and the spindle poles (centrosomes) migrate apart.

During **telophase**, the daughter chromosomes arrive at the spindle. The **nuclear envelope reassembles** at each pole and there is assembly of the **contractile ring**.

**Cytokinesis** is cell division. Microtubules pulled chromosomes to opposite sides of the cell. Contraction of the **acto-myosin ring** occurs and you get a midbody beginning to form and a new membrane is inserted. The chromatin decondenses and nuclear substructures reform. Two daughter cells are formed.

**The Mitotic Checkpoint**

At the transition out of metaphase, there is a **spindle assembly checkpoint**. This senses the completion of chromosome alignment and spindle assembly (monitors kinetochore activity). The **mitotic checkpoint** is active between prometaphase and metaphase.

After duplication of the chromosomes, they all need to be picked up by the spindle so they can be pulled. When the kinetochores have not attached to the spindle, they keep **signalling** so the cell cannot proceed. As soon as a microtubule is attached, the signal stops and the cell and continue in the cycle. There are a number of proteins that regulate this, two examples are **CENP-E** and **BUB protein kinase**. BUBs dissociate from the kinetochore when chromosomes are properly attached to the spindle. When they have all dissociated, anaphase proceeds.

**When this is not regulated properly**, the cells lose or gain a whole set of chromosomes. **Aneuploidy** results by two main pathways.

The first is when there is **mis-attachment of microtubules to kinetochores**. Normal attachment of a chromosome is called **amphelic** attachment. When there is misalignment of the chromosomes, you could get two sister chromatids linked to the same pole. This pair will go to just one daughter cell. This is called **syntelic** attachment. The same can happen if only one chromatid is attached, and the other will just float about. This is called **monotelic** attachment. In any of these situations the daughter cells will have an abnormal number of chromosomes. Another thing that can happen is that the kinetochore of a chromatid could be attached to a microtubule from both centrosomes. So this kinetochore will say its alright to proceed, but the cell starts dividing, and the chromosome is either broken or lost in cell division. This is called **metrotelic** attachment.

The second road to aneuploidy is where there is an **aberrant mitosis**. There could be an aberrant cell cycle and aberrant DNA and chromosome duplication. So instead of two poles, there could be four poles, all of them producing microtubules competing for sister chromatids. The cell divides four ways, and a couple of cells might not even have any chromosomes. This is quite serious, and these cells usually die.
Anti-cancer therapy can play into these processes. Tumours proliferate so fast, it is good to target cells when they are vulnerable. One possibility is to inhibit the attachment of the error-correction mechanism (mitotic checkpoint). Another possibility is a checkpoint kinase inhibitor, so the chromatids signal that the kinetochores have all attached when actually they haven’t. This leads to aneuploidy, making the cell inviable = apoptosis. Taxanes and vinca alkaloids are used in breast and ovarian cancers. These drugs alter microtubule dynamics, by stopping the cells forming spindles properly. This produces unattached kinetochores, causing long-term mitotic arrest.

When the cells finish mitosis, they have two options. If further proliferation is required, they go directly on to the G1 phase. In this case there will be a check of whether there are appropriate conditions for cell division (nutrients, growth factors in environment). If no proliferation is required, then the cells are segregated in the G0 phase where they exit the cell cycle and they dismantle their cell cycle apparatus. The cells become active in their tissues. They can also now differentiate, but this requires different signals.

Summary of Cell Cycle Checkpoints

In the absence of a stimulus, the cells go into the G0 quiescent phase. Most cells in the body which are differentiated to perform specific functions are in the G0 phase, but they are not dormant, they are all actively doing their functions. To exit G0, the cell needs a stimulus, which is usually growth factors and intracellular signalling cascades.

When growth factors bind to the cell, levels of a protein called Myc increase very quickly, and this is one of the triggers for the cell to go into the G1 phase.

Once the cell senses in the environment that everything is alright for division, the receptor is activated, and there is a signalling cascade which amplifies this signal. The response to extracellular factors causes signal amplification, signal integration, there is modulation by other pathways, and regulation of divergent responses.
With signalling by peptide growth factors, the ligand binds to and activates the receptor. An example of how growth factors stimulate their receptor is EGF (epidermal growth factor). This is a highly amplified receptor in tumour cells. Respective receptors are found in their monomeric inactive state. In the presence of a ligand, the receptors form dimers. These receptors usually have a kinase domain in their tails, which is why they are known as receptor protein tyrosine kinases (RPTK). As they dimerise, the kinase domains are activated by phosphorylation.

**Protein phosphorylation** usually involves the transfer of a phosphate from ATP to a hydroxyl group on a protein. These OH groups may be serine residues, threonine residues, or tyrosine residues. The added phosphate group (negatively charged) can alter protein function by causing a change in conformation leading to a change in activity, or by creating a docking site for another protein. Phosphatases cleave the phosphates out again.

An example of a conformation change is signalling by peptide growth factor. In the presence of the ligand, the receptors form dimers and are activated by phosphorylation. Receptor activation triggers kinase cascades and binding of adapter proteins. In a kinase cascade, the protein regulated by a kinase is another kinase, and so on. This leads to signal amplification, diversification and the opportunity for regulation.

**Adapter proteins** can recognise phosphorylated amino acids, and so once they are phosphorylated a whole series of adapter proteins can bind to the tail of the receptor, which modulates function.

**Tyrosine phosphorylation** provides docking sites for adapter proteins. Proteins are modular and contain domains, i.e. functional and structural units that are copied in many proteins. Some domains are important in molecular recognition. For example, Grb2 is a protein containing 3 domains (Src Homology regions - two SH3 domains and one SH2 domain. They recognise phosphorylated regions. SH3 recognise proline-rich (constitutive) regions, and SH2 recognises phosphorylated tyrosines (inducible, specific sequence context).

What if something goes wrong during the cell cycle? For example if the cell is not big enough or there is DNA damage? There are two options.

**Cell cycle arrest** happens at the check points (G1 and spindle check point). This can be temporary, as if the cell can repair the DNA damage the cycle will continue. If not, cell cycle progression is aborted and the cell is destroyed.

The other option is **programmed cell death - apoptosis**. This happens if DNA damage is too great and cannot be repaired, there are chromosomal abnormalities, or if there are toxic agents. Cell cycle progression is aborted and the cell is destroyed.

**Learning Objectives for this lecture:**
- Describe the cell cycle in terms of the named phases and explain what these mean in terms of protein and DNA synthesis.
- Identify the named stages of mitosis.
- Explain the importance of checkpoints in controlling progression through the cell cycle, and give examples of external factors which provide signals allowing cells to pass these checkpoints and enter cell division.
- Describe the way the cell cycle allows decision making about whether a cell divides, differentiates or undergoes programmed cell death.
- Introduce the principle of molecular timing processes which regulates the cell cycle through oscillating amounts of activities of cyclins and their kinases.
Most adult cells are not constantly dividing. In the absence of growth signals they go into the GO, or quiescent, phase (e.g. liver hepatocytes). Entry into the cell cycle is the key role of a transcription factor called Myc. In the quiescent GO phase, the level of Myc is very low. As soon as growth factor is present, the level of Myc rapidly rises and plateaus at a substantial level of expression.

Key components of signalling pathways include regulation of enzyme activity by protein phosphorylation (kinases), adapter proteins, and regulation by GTP-binding proteins.

**Mitogenic growth factor** (i.e. growth signals from other cells e.g. Hepatocyte Growth Factor released after liver damage) binds to the receptor protein tyrosine kinase, which is G-protein linked (Ras). There is then the kinase cascade and downstream signalling. Immediate early genes (c-jun, c-fos, c-myc - transcription factors) stimulate the expression of other genes.

In signalling by peptide growth factors, the phosphorylated receptor recruits adaptor and signalling proteins (e.g. Grb2). Anti-HER2 Ab Herceptin is used to block the early phase of signalling in breast cancer by acting at the tyrosine kinase receptor. Grb2 recruits an exchange factor called Sos, which activates a small signalling protein called Ras, which then passes on the signal. Ras must bind to the plasma membrane to become activated, so another possible target to block the signalling pathway is the binding of Ras to the plasma membrane.

Ras is an example of GTP binding G-proteins. These proteins sit in the cell bound to GDP in the inactive conformation. The exchange protein Sos causes exchange of GDP for GTP from the cytoplasm, causing a conformational change = activated. Active Ras is able to propagate the signal. The signal is regulated by the intrinsic ability of Ras to hydrolyse the GTP back to GDP to turn itself off again slowly. The cell uses a family of proteins called GTPase Activating Proteins (GAPs) to help this regulatory process.

Ras can be oncogenically activated by mutations that increase the amount of active GTP loaded Ras. One common mutation is where glycine at position 12 is changed into valine. This prevents GAP binding, and so this prevents inactivation. Another mutation is where glutamine 61 changes to leucine. This prevents GTP hydrolysis. In both of these mutations, there is a higher proportion of GTP-bound Ras, which promotes cell division and cancer.

GTP-bound active Ras stimulates a protein kinase cascade, which involves three types of kinases. Each kinase in the pathway uses ATP to phosphorylate the next kinase down in the pathway. In the case of growth factor stimulated signalling, the pathway is called the extracellular signal-regulated kinase (ERK) cascade. This particular paradigm of kinase cascade is used by cells in many different signalling situations. The family of cascades are generically called mitogen activated protein kinase (MAPK) cascades.

The kinase at the bottom of the ERK cascade (kinase III) is called ERK, the one that phosphorylates that one (kinase II) is called MEK, and the one that phosphorylates that one (kinase I) is called Raf. In its active conformation, Ras binds to Raf, causes it to become activated and phosphorylate MEK, and MEK phosphorylates ERK.
Protein kinases stimulate changes in cell proteins and gene expression to promote division. ERK phosphorylates target proteins to push the cell through the cycle. ERK can turn proteins on and off, and also influences gene expression for cell cycle progression. One of those genes that is turned on by ERK is Myc. Two very important oncogenes are Myc and Ras.

Cell division is a very complex process, and it requires strict regulation. This is why there are checkpoints as the cell cycle progresses. These checkpoints are dependent on cyclically activated protein kinases. They come on at certain points in the cell cycle to allow progression. These kinases are called cyclin dependent kinases (CDKs). They are present in proliferating cells throughout the cell cycle. Their activity is regulated by interaction with cyclins and by phosphorylation.

Cyclins are transiently expressed at specific points in the cell cycle. They are regulated at the level of expression. They are synthesised, activate CDKs and then are degraded before the cell moves on to the next stage. There are different cyclin-CDK complexes that trigger different events in the cell cycle.

In mitosis, the CDK is CDK1. It binds to and is activated by a mitotic cyclin (only produced during mitosis). CDK1 + mitotic cyclin = mitosis promoting factor (MPF). Cyclic synthesis and degradation of cyclins is very important in the regulation of the cell cycle.

Phosphorylation is also important in regulating CDKs. There are two kinases involved in controlling this, there is CDK activating kinase (CAK) and there is an inhibitory kinase (Wee1). The activating kinase activates one site on CDK to promote its activity. However, if the other site is activated by Wee1, then this suppresses its activity. In order for it to be catalytically active, the inhibitory phosphorylation has to be removed by a phosphatase called Cdc25.

So regulation of CDKs by phosphorylation requires the activating phosphorylation and removal of the inactivating phosphorylation. Cdc25 itself is under the control of phosphorylation, and is one of the substrates of active MPF. There is a positive feedback effect, which reinforces the activation of MPF and drives mitosis.

Different cyclins and different CDKs are required at different stages of the cell cycle. Cyclins activate CDKs and alter substrate specificity, and so substrate accessibility changes through the cell cycle.

Growth factor stimulation of signalling pathways promotes G0 to G1 transition. This turns on the transcription factor Myc, which induces the production of cyclin D. This is specifically active at the phase of driving cells from G0 into the cell cycle. Cyclin D activates CDK4 and CDK6 to stimulate the synthesis of cyclin E in the G1 phase.

CDKs become sequentially active and stimulate synthesis of genes required for the next phase of the cycle. This gives the cycle direction and timing. Cyclins are of course susceptible to degradation, and hence there can be cyclical activation.

CDKs phosphorylate proteins (on Serine of Threonine) to effect cell cycle progression. CDK1 (dependent on mitotic cyclins like cyclin B) drives mitosis, as nuclear laminins of MPF cause the breakdown of the nuclear envelope. CDK2 (dependent on cyclin E) drives the G1 phase, by phosphorylating targets like the retinoblastoma protein (pRb).

pRb is a large protein which sits in cells. In a resting G0 cell, it is unphosphorylated, but it can bind to and sequester a family of transcription factors (E2F). The pRb protein is a target of cyclin dependent kinases. CDK4 and CDK6 cyclin D (which drives the cell from G0 into G1) and also CDK 2 cyclin E (which moves cells through
the G1/S phase) can all phosphorylate the pRb at multiple sites. As this happens, pRb’s affinity for E2F is reduced, so E2F is released and it can induce target genes like the cyclin E gene.

pRb acts as a brake on the cell cycle, and CDKs inactivate pRb. Rb is a “tumour suppressor”, which is lost in many cancers.

The E2F family of transcription factors regulate many genes in the cell cycle, for example Myc, pRb, cyclins, CDKs, and themselves. They also regulate many components involved in DNA synthesis like thymidine kinase and DNA polymerase.

Myc induces cyclin D, which turns on CDK4/6 as a complex. This starts to phosphorylate pRb, which releases some E2F, which is then able to bind to the cyclin E promoter and start its production.

Cyclin E complexes with CDK2, which can then further phosphorylate pRb, which can then release more E2F, which then stimulates the promoter of cyclin A...

This process of hyperphosphorylation of the regulatory proteins and the release of transcription factors is carried out throughout the cell cycle.

CDK inhibitors (CKIs) regulate CDKs. These can bind to and inactivate CDK-cyclin complexes.

There are two families of CKIs. The INK family are active in the G1 phase to inhibit CDK4/6. The CIP/KIP family are active in the S phase and inhibit all CDKs. They must be degraded to allow cell cycle progression. The INK family work by displacing cyclin from the CDK complex = inactivation. The CIP/KIP family work by binding to the whole complex and inactivating it.

Proteins involved in regulating the cell cycle are extremely important in cancer. Oncogenes like EGFR/HER2 are mutationally activated or over expressed in many breast cancers (Herceptin antibody for the treatment of HER2-positive metastatic breast cancer). Ras is mutationally activated in many cancers (inhibitors of membrane attachment for treatment). Cyclin D1 is over expressed in 50% of breast cancers. B-Raf is mutationally activated in melanomas (kinase inhibitors in trials for treatment). c-Myc is also over expressed in many tumours.

Tumour suppressors include Rb, which is inactivated in many cancers. p27KIP1 under expression correlates with poor prognosis in many malignancies.

Learning Objectives for this lecture:
- Explain how ligands which activate tyrosine kinase receptors signal through the small G protein, Ras, to activate the extracellular signal-regulated kinase (ERK) cascade.
- Describe how the ERK cascade pathway regulates gene expression and leads to progression through G1 of the cell cycle.
- Outline the principle of the molecular timing process which regulates the cell cycle through oscillating amounts or activities of cyclins, their kinases and inhibitor proteins.
**Apoptosis**
by Professor Tony Magee

Programmed cell death is important for a number of reasons. We need to get rid of harmful cells (e.g. cells with viral infection or DNA damage), developmentally defective cells (e.g. B lymphocytes expressing antibodies against self antigens), excess cells (e.g. during embryonic development brain to eliminate excess neurons, liver regeneration, sculpting of digits and organs), obsolete cells (e.g. mammary epithelium at end of lactation), and it is also important in the chemotherapeutic killing of cells.

Necrosis = unregulated cell death associated with trauma, cellular disruption and an inflammatory response.

Apoptosis = regulated cell death, controlled disassembly of cellular contents without disruption, no inflammatory response.

In necrosis, the plasma membrane becomes permeable, which results in cell swelling and rupture of cellular membranes. This leads to the release of proteases = autodigestion and dissolution of the cell = local inflammation.

Cell Death
Necrosis - unregulated cell death associated with cellular disruption and an inflammatory response.

Apoptosis - (programmed cell death) - regulated cell death, controlled disassembly of cellular contents, no inflammatory response.

Apoptosis-like programmed cell death - some, but not all, features of apoptosis. Display of phagocytic recognition molecules before plasma membrane lysis.

Necrosis-like programmed cell death - variable features of apoptosis before cell lysis, “aborted apoptosis”.

Features of Apoptosis
In the latent phase, death pathways are activated, but cells appear morphologically the same. In the execution phase, there is loss of microvilli and intercellular junctions, cell shrinkage and loss of plasma membrane asymmetry (phosphatidylserine lipid appears in outer leaflet). In the nucleus there is chromatin and nuclear condensation and DNA fragmentation. There is the formation of membrane blebs, and fragmentation into membrane-enclosed apoptotic bodies. The plasma membrane remains intact.

DNA modification in apoptosis is seen as nuclear condensation and DNA fragmentation. The chromosomes are broken in a very regulated way by specific nucleases. As apoptosis proceeds, you can see a “laddering” effect on agarose gel. DNA fragmentation can be seen by the TUNEL staining assay technique. It is seen that fragmentation leads to more “ends” which are labelled by adding an extra tagged base that is detected with a fluorescent probe.

In the later stages, the cells shrink and you can see membrane blebbing under the microscope. There is loss of microvilli and cell shrinkage. There is then phagocytosis of apoptotic bodies by surrounding cells like macrophages.

Mechanisms of Apoptotic Cell Death
The executioners are caspases. These are cysteine-dependent aspartate-directed proteases, and they are the executioners of apoptosis. Caspases are activated by proteolysis, which triggers a cascade of activation. There are two types: initiator caspases and effector caspases. Effector caspases (3, 6, and 7) have a similar molecular organisation. They start off as a single chain polypeptide, but within this are two subunits released by proteolytic cleavage. The initiator caspases (2, 8, 9 and 10) have that same pair of modules, but in addition they have an extra targeting subunit which directs them to a particular location. Some have a targeting subunit called a CARD (caspases recruitment domain), and some have a DED (death effector domain).

Caspases start off as single chain polypeptides. In this form they are inactive, sitting around in cells (zymogens). To be activated they have to undergo proteolytic cleavage, triggered by death pathways. They
are cleaved to form a large and small subunit, and the initiator caspases also release the targeting subunits. These cleavages are done by the caspases themselves. Once apoptosis is initiated, the **initiator caspases cleave the downstream effector caspases**, which then go on to carry out the apoptotic programme.

Effector caspases execute the apoptotic programme by cleaving and inactivating various proteins and complexes (e.g. nuclear lamins leading to nuclear breakdown). The **effector caspases activate enzymes** (including protein kinases, nucleases like Caspase Activated DNase) by direct cleavage or cleavage of inhibitory molecules.

Caspase activation is done in two main ways. One is a receptor-mediated pathway (extrinsic), which is called **death by design**. The other is an intrinsic pathway dependent on the integrity of mitochondria, which is called **death by default**.

**Death by Design**

Essentially all cells have this type of “death receptor” on their surface. They are ready to receive signals which tell the cell to die under the appropriate circumstances, e.g. TNF or Fas. The diagram shows receptors for various ligands which are waiting for the cell to get the death signal. A single transmembrane protein is characterised by how many cysteine rich domains they have in the extracellular portion, and they have a domain in the cytoplasmic portion called the death domain.

Two **adapter proteins** involved in this receptor mediated pathway are **FADD**, which is a positive regulator (required for the death pathway to be activated), and **FLIP**, which is a negative regulator (inhibits the death pathway and allows it to be regulated). FADD contains two domains: DED and DD (Death Domain). FLIP just contains two DED domains.

Taking Fas as an example of a death receptor, it is often up-regulated if the cell is e.g. infected with a virus. Fas can be engaged by **Fas ligand (Fas-L)**, which is on the surface of cytotoxic T lymphocytes. If a T cell sees a cell with a high level of Fas on its surface, the Fas-L will engage the receptors on the cell. In the process of binding, it causes the Fas receptor to **trimerise** (bring 3 receptors into close proximity). This brings 3 DED domains close together. At this point, the positive regulator of apoptosis (FADD) is recruited to these DDs by its own DD. Subsequently, **pro-caspase 8** (an initiator caspase) is recruited to the FADD DD by its own DED. A complex is built up - the **Death Inducing Signalling Complex (DISC)**.

Because there is a trimer, three initiator pro-caspase molecules can be recruited to the DISC. There is then **cross-activation** of the pro-caspases, whereby they **cleave** each other within that complex because they have been brought into close proximity of each other. The active **caspase 8** is then released, and it cleaves effector caspases to carry out the death programme.

Pro-caspases are essentially inactive, but they can cleave each other in this case. There are two hypotheses to explain this. One is that they are not actually completely inactive, they have a very low activity, so when you bring three of them into close proximity they can cleave each other. The other idea is that recruiting the pro-caspase to the membrane in this way causes a conformational change which imparts some activity. The bottom line is that bringing the three pro-caspases close together triggers the apoptotic programme.
The negative regulator FLIP comes in a short form and a long form. FLIP looks very much like a caspase, as it has two DEDs and the long form even has caspase homology domains. But FLIP is completely inactive and has no catalytic activity like a caspase. What it does have, is the DDs, which allow it to bind to FADD in the DISC. FLIP can bind to the FADD with no activity, and so it can compete for binding with the pro-caspase 8 and reduce its activity. FLIP incorporates into receptor-pro-caspase complexes and interferes with transcleavage. The balance between FLIP and positive signalling in the receptor determines whether the death programme is carried out or not.

Death by Default
The second major pathway in cell death by apoptosis involves mitochondria - the intrinsic pathway. A number of cellular stresses (e.g. lack of growth factors, DNA damage, etc) can perturb the mitochondrial transmembrane potential. The mitochondrial membrane potential collapses, and that allows cytochrome C (major soluble mitochondrial component) to be released into the cytoplasm, where it wouldn’t usually be, so this signals to the cell that the cell’s energy production system is compromised = die. The cytochrome C that is released becomes incorporated into another molecular machine which can trigger the death pathway - the apoptosome.

The apoptosome (wheel of death) is made up primarily of one very large protein (Apaf1 = apoptotic activating factor-1)) which sits around in the cytoplasm, waiting to get a signal. At one end, Apaf1 contains a number of repeats which are involved in protein-protein interactions. At the other end there is an ATPase domain. At the front there is a caspase recruitment domain (CARD), which is also found in some initiator caspases (e.g. caspase 9).

When cytochrome C binds to the WD-40 repeats on Apaf1, it triggers a conformational change in the protein which causes it to assemble into a heptameric structure with cytochrome C bound to the arms. The assembly of this heptameric molecular machine requires ATP, so ATP is required for this death pathway. The CARD domains at the centre of the heptamer are capable of interacting with the CARD domains on pro-caspase 9. Therefore, pro-caspase 9 is recruited to the heptamer by its own CARD domain and it binds. Each Apaf1 in the heptamer can potentially bind a pro-caspase 9. Oligomerisation brings multiple pro-caspase 9s into close proximity of each other, which means they can cross-cleave each other to produce active caspase 9, which can be released and trigger the cleavage of effector caspases and the death pathway = apoptosis.

So there is the receptor mediated mechanism which activates caspase 8, and the mitochondrion mediated mechanism which activates caspase 9. Both of these initiator caspases are capable of cleaving effector caspases to trigger the apoptotic pathway.

There is another mechanism that re-enforces the receptor mediated pathway by bringing in the mitochondrial pathway too. This really pushes the cell through to death. This is done by a protein called Bid. Bid can be cleaved by caspase 8. Once it is cleaved, it can go to the mitochondria and promote the release of cytochrome C. So the receptor mediated pathway recruits the mitochondria mediated pathway shortly after.

The fact that the apoptosome requires ATP for its formation, may somewhat explain the differences between different types of cell death. Energy levels in the cell may determine whether death is by necrosis or apoptosis. In a cell where there is plenty of ATP then this favours apoptosis. If cellular ATP levels are low, then you can’t engage the mitochondrial apoptotic pathway, so cells will tend to die by necrosis.
Regulators of the Apoptotic Programme

Members of the Bcl-2 family like Bid have different structures, some are transmembrane proteins, some are bigger cytoplasmic proteins. The thing they have in common is a BH3 domain, which is a protein-protein interaction motif, which allows proteins of this family to associate and dimerise with each other.

Members of this family fall into two categories. Some are anti-apoptotic (inhibit apoptosis, like Bcl-2 and Bcl-xL), and these proteins localise to the mitochondrial membrane. Other members are pro-apoptotic (promote apoptosis, like Bid, Bad and Bax), and these have the ability to move between the cytosol and the mitochondria.

Bid is the protein which links the receptor mediated apoptosis pathway to the mitochondria mediated apoptotic pathway.

Growth factor receptors activate the Ras ERK pathway, controlling the cell cycle and cell growth. Tyrosine kinase receptors get phosphorylated multiple times. One of the docking sites created by phosphorylation is for Grb2, which triggers the Ras pathway. Another phosphorylation site triggers a different pathway involved in cell survival and anti-apoptotic effects - the PI3'-Kinase Pathway.

One phosphorylation site recruits the kinase enzyme PI3'-Kinase. It is a lipid kinase involved in growth control and cell survival. It has a targeting subunit, an adapter subunit and a catalytic subunit. It is recruited to the activated receptor, and it then phosphorylates a substrate. The substrate is a lipid in the plasma membrane called PIP2. PI3'-Kinase phosphorylates the 3 position to create PIP3. This is recognised by the adapter subunit of PKB (protein kinase B), which is an enzyme that is recruited to the membrane and becomes activated. It is an anti-apoptotic enzyme.

PKB phosphorylates one of the Bcl-2 family proteins called Bad, and causes it to be held in an inactive complex in the cytoplasm. Normally, when cells have plenty of growth factor, this pathway is activated and Bad is held as an inactive complex in the cytoplasm. The outer membrane of the mitochondria contains other members of the Bcl-2 family. There are anti-apoptotic members like Bcl-2 and Bcl-xl, and there are pro-apoptotic members like Bax and Bad. The anti-apoptotic members hold the pro-apoptotic members inactive by binding to them by their BH3 domains.

When there is not much growth factor or the signalling pathway is switched off, PKB activity drops, phosphatase enzymes in the cell are able to remove the phosphate from Bad, and so Bad becomes de-phosphorylated, it is released from its binding protein, and it then goes into the mitochondria where it can bind through its own BH3 domain to the BH3 domains of the anti-apoptotic Bcl-2 family members and displace the pro-apoptotic Bcl-2 family members.

Once the pro-apoptotic family members are released from inhibition, they form a pore in the mitochondrial membrane, which allows cytochrome C to escape into the cytosol.

So Bcl-2 family members are crucial in keeping the mitochondrial death pathway suppressed. But when there is an appropriate stimulus or there is removal of growth factor, this pathway can become activated and the cell is triggered to die. PTEN (lipid phosphatase) counteracts PI3'-Kinase signalling. PTEN is a tumour suppressor. It promotes the dephosphorylation of PIP3 back to PIP2 and antagonises that pathway.

PKB plays a crucial role in maintaining cell survival, not only by phosphorylating Bad. It can also phosphorylate and inactivate caspase 9 directly. It can inactivate a family of FOXO transcription factors that promote the expression of apoptotic genes. It also has other effects like stimulating protein synthesis.

There is yet another level of regulation by Inhibitors of Apoptosis Proteins (IAPs), and they work primarily by regulating caspase activity. They bind to pro-caspases and prevent activation. They also bind to active caspases and inhibit their activity.
So in summary, pathways that control apoptosis include the **Bcl-2 family members** that control the intrinsic mitochondria mediated pathway, and there are **FLIP and IAPs** that regulate the extrinsic receptor mediated pathway. There are also growth factor pathways via **PI3'-K and PKB/Akt** which regulate cell death.

How does all this relate to cancer? If **Bcl-2** is over-expressed, then it is anti-apoptotic and will promote cell survival, so it can actually act as an **oncogene** in many cancers. Currently, small molecule mimics of the BH3 domain are being developed as anti-cancer agents to displace Bcl-2 from the pro-apoptotic family members. **PKB/Akt** also has anti-apoptotic effects and so can be an **oncogene** in certain types of tumour if it is over-expressed. **PTEN** on the other hand (because it opposes the activity of the PI3'-K pathway) is pro-apoptotic and can act as a **tumour suppressor**. It is deleted or inactivated in many cancers.

The uses of programmed cell death for humans include **removal of harmful** (oncogenic) **cells** or cells with viral infections or DNA damage. It can also be taken advantage of in **chemotherapeutic killing** of cells. **Dexamethasone** works by promoting apoptosis.

**Learning Objectives for this lecture:**
- Explain the difference between necrosis and apoptosis and describe how they may be differentiated.
- Discuss whether necrosis and apoptosis are the only forms of cell death.
- Describe the proteolytic caspase cascades which execute the apoptotic response.
- Discuss how apoptosis may be mediated through death receptors and/or the mitochondria.
- Discuss how Bcl-2 family proteins may modulate apoptosis.
External Factors Controlling Cell Division and Cell Behaviour
by Dr Peter Clark

Many external influences are detected by cells. Chemical influences include hormones, growth factors, ion concentrations, extracellular matrix, molecules on other cells, nutrients and dissolved gas concentrations. Physical influences include mechanical stresses, temperature, the topography or “layout” of the extra-cellular matrix and other cells.

Although all external factors may potentially influence cell proliferation, the best understood, and the ones to be concerned about with regards to cell division are: growth factors, cell-cell adhesion and cell-ECM adhesion.

Cell-ECM adhesion was the focus of experiments where the rate of cell division was compared in cells suspended in agar (non-adhesive environment) and cells perched on a small adhesive patch (made using micro-fabrication technology). Growth factors were present throughout.

What this experiment showed was that a very low frequency of cells divided in suspension. A much higher frequency divided on the adhesive patch. Cells require to be binding to extracellular matrix to be fully competent for responding to soluble growth factors.

In suspension, cells do not significantly synthesise protein or DNA. Cells require to be attached to extra-cellular matrix (and a degree of spreading is required) to begin protein synthesis and proliferation (DNA synthesis). Attachment to ECM may be required for survival (e.g. epithelia, endothelia). This is known as anchorage dependence.

Cells have specific receptors on their cell surface which bind to extra-cellular molecules. These molecules are often linked, at their cytoplasmic domains, to the cytoskeleton. This arrangement means that there is mechanical continuity between extra-cellular matrix and the cell inferior.

Integrins are heterodimer complexes of α and β subunits that associate extracellularly by their “head” regions. Each of the “tail” regions spans the plasma membrane.

Integrins are the most important ECM receptors. They recognise short, specific peptide sequences (e.g. α5β1 fibronectin receptor binds arg-gly-asp RGD). There are more than 20 combinations of αβ integrin molecules known. Each combination specifically binds a particular peptide sequence. Such peptide sequences are often found in more than one extra-cellular matrix molecule, e.g. RGD is found in fibronectin, vitronectin, fibrinogen plus others.

Most integrins are linked, via actin binding proteins, to the actin cytoskeleton. The α6β4 integrin complex is found in epithelial hemidesmosomes, linked to the cytoskeleton (intermediate filament) network. Integrin complexes cluster to form focal adhesions (most) or hemidesmosomes (α6β4). These clusters are involved in signal transduction. Integrins also bind to specific adhesion molecules on some cells (e.g. α,β3 binds to PECAM-1 (CD31) and α11β2 to ICAM-1 on endothelial cells in inflammation).

Extra-cellular matrix receptors (e.g. integrins) can act to transduce signals, for example ECM binding to an integrin complex can stimulate the complex to produce a signal inside the cell, i.e. “outside-in signalling”.

A signal generated inside the cell (e.g. as the result of hormone binding to receptor) can also act on an integrin complex to alter the affinity of an integrin (i.e. alter its affinity for its ECM binding). This “inside-out” signalling is important in inflammation or blood-clotting, or switching on adhesion of circulating leukocytes.
In “outside-in” signalling, a cell can receive information about its surroundings from its adhesion to ECM, e.g. the composition of the ECM will determine which integrin complexes bind and which signals it receives. This can alter the phenotype of the cell.

Integrin activation and signalling involves significant conformational changes to the complex. “Inside-out” activation extends the flexed complex. Ligand-binding opens the legs of the complex allowing cytoplasmic signalling molecules to bind - “outside-in” signalling.

Integrins recruit cytoplasmic proteins which promote both signalling and actin assembly.

There were some very elegant experiments done in the early 90s where mammary epithelium was cultured in gels of matrix proteins. When the gel was made up of the matrix found in interstitium (type 1 collagen), the epithelium formed clumps, it did not differentiate to secretory cells. In a gel made up of laminin resin (basement membrane basal lamina matrix), the epithelial cells organised themselves into hollow cysts (“organoids”), and then started to produce milk proteins.

At high density, cells compete for growth factors. When cells in culture form a confluent monolayer, they cease proliferating and slow down many other metabolic activities. This used to be known as contact inhibition of cell division. Another set of experiments suggest that it is competition for external growth factors and not cell-cell contact responsible - density dependence of cell division.

One of the major stimuli for growth is a soluble growth factor. One of the most common signalling pathways for growth factor stimulation is the ERK/MAPK pathway.

The growth factor binds to the receptor (in this case tyrosine kinase receptor). Binding causes the receptor to recruit proteins (adapters) which in turn recruit other proteins and stimulate the signalling cascade. The cascade in this case is raf, MEK and ERK. These are all kinases. When ERK is activated, it can do a number of things. Some of this is directly related to changes in gene expression in the nucleus.

There is also cross-talk between the extra-cellular matrix and growth factor signalling. Even if growth factors are present, cells need to be attached to matrix to be competent for cell division.

The signalling pathways from growth factor and from the extracellular matrix must converge onto the same pathway to get proliferation. This is the combination of density dependence and anchorage dependence. Growth factor receptors and integrin signalling complexes can each activate identical signalling pathways (e.g. MAPK). Individually, this activation is weak and transient. Together, activation is strong and sustained. The separate signalling pathways act synergistically.

There are two types of contact interactions between cells. There are short-term, transient interactions between cells which do not form stable cell-cell junctions. There are also long term, stable interactions resulting in formation of cell-cell junctions.

When most non-epithelial cells “collide”, they do not form stable cell-cell contacts. They actually “repel” one another by paralysing motility at the contact site, promoting the formation of a motile front at another site on the cell, and moving off in the opposite direction. This is contact inhibition of locomotion and is responsible for preventing multi-layering of cells in culture and in vivo.
In terms of long-term cell-cell contacts, upon contact, some cell types strongly adhere and form specific cell-cell junctions (adherens junctions, desmosomes, tight junctions, gap junctions). This is true of epithelial cells and endothelial cells which form layers, and neurones forming synapses.

Cell-cell junctions in epithelia are usually arranged as continuous belts (zonula) or as discrete spots (macula). Contact between epithelial cells leads to the mutual induction of spreading, so that the total spread area of the contacted cells is greater than that of the sum of the two separated cells. This could result in a stable monolayer.

Cell-cell adhesion affects cell proliferation. When there are no cell-cell junctions, activated MAPK, and decreased p27KIP1, there is high proliferation. This is the case when there is a low concentration of calcium ions. When there are cell-cell junctions, inactive MAPK, and increased p27KIP1, there is low proliferation. Again, this is likely to be calcium dependent - when there is a high concentration of calcium.

Adhering junctions are mediated by cadherins. They are the molecules that span the plasma membrane and associate with identical molecules on adjacent cells. They are like the teeth of a zip.

The adhesion molecule of the cadherin spans the membrane. Intracellularly, it associates with a β-catenin molecule, which associates with an α-catenin molecule, which associates with an actin filament. The question is: is β-catenin the link between cell-cell adhesion and proliferation? In adenomatous polyposis coli (APC, an inherited colon cancer), the APC gene-product is a protein involved in the degradation of the junction-associated molecule β-catenin. There is overgrowth of the colon epithelium, resulting in thousands of polyps. This is indicative of a hugely elevated rate of cell proliferation.

This happens because β-catenin can associate with another molecule called LEF-1, go into the nucleus and act as a transcription factor.

The APC gene is part of a complex with a glycogen synthase kinase molecule that phosphorylates β-catenin. When this does so, it targets it for degradation. If β-catenin hangs around in the cytoplasm, it gets targeted with LEF-1, goes into the nucleus, and switches on proliferation.

When bound to cadherin at the membrane, β-catenin is not available for LEF-1 binding and the consequent nuclear effects. Normally, cytoplasmic β-catenin is rapidly degraded in the cytoplasm. If β-catenin cytoplasmic levels rise as a result of inhibition of degradation or loss of cadherin-mediated adhesion, β-catenin/LEF-1 complex enters the nucleus and influences gene expression, leading to proliferation.

Other adhesion-associated signalling pathways are known to influence contact induced inhibition of proliferation. Clustering of cadherins after cell-cell contact is known to alter the activation of small GTPases, e.g. Rac is activated, Rho is inhibited, and this can influence proliferation. Some growth factor receptors are associated with cell-cell junctions by being incorporated into them.

Cancer

Under certain conditions, cells lose their behavioural restraints. As a result, they do a number of things. They proliferate uncontrollably (lose density dependence of proliferation), they are less adherent and will multilayer (lose contact inhibition of locomotion and anchorage dependence), epithelia breakdown cell-cell contacts (less constrained), and they are not Hayflick limited - they express telomerase.

Normal contact-inhibited cells form a stable monolayer of cells. Cancer cells form multi-layered uninhibited cells. Cancer cells are not contact-inhibited.
If the gene coding for a component of a signalling pathway is mutated so that the protein is constitutively active, that pathway will be permanently 'on'. Receptors, signalling intermediates, and signalling targets (e.g. transcription factors) are proto-oncogenes. This is the mechanism of short-circuiting leading to uncontrolled proliferation as a result of loss of growth factor dependence and a loss of anchorage dependence.

**Oncogene** = mutant gene which promotes uncontrolled cell proliferation. **Proto-oncogene** = normal cellular gene corresponding to the oncogene. Ras is a signalling intermediate part of the MAP kinase pathway, as is the signalling intermediate c-Raf. V12Ras is an oncogene example of Ras, and v-Raf is an oncogene example of Raf. c-Jun is a transcription factor, and v-Jun is an oncogene example.

Ras is mutated in about 30% of all cancers.

Adenomas are **benign** tumours tend to be encapsulated and are not aggressively invasive. Adenocarcinomas are **malignant** tumours which are invasive and metastasise. In addition to deregulated proliferation, a major feature of cancerous tumours is their ability to spread. Most human cancers are **carcinomas** (i.e. of epithelial origin). In order to spread to other site (metastasis), cells must break away from the primary tumour, travel to a blood or lymph vessel, enter the vessel, lodge at a distant site, leave the vessel, and ultimately establish a secondary tumour. **Cell-cell adhesion** must be down-regulated (e.g. cadherin levels reduced), and the cells must be motile. Degradation of the ECM must take place, and matrix metalloproteinase (MMP) levels are increased in order to migrate through basal lamina and interstitial ECM. The degree of carcinoma cell-cell adhesion is an indicator of how differentiated the primary tumour is, and indicates its **invasiveness** and the **prognosis**.

**Learning Objectives for this lecture:**

- Describe using examples the role of external growth factors in controlling the decision of a cell to divide.
- Describe using examples how mutation of a proto-oncogene can perturb the normal controls on cell division.
- Explain why signalling pathways involving growth factors are often implicated in the uncontrolled division of cancerous cells.
- Explain the role of contact inhibition and anchorage dependence in limiting the division of normal cells within their tissues.
- Discuss the factors which restrain cells within their normal tissue boundaries.
The Cytoskeleton
by Professor Mike Ferenczi

There are three major components of the cytoskeleton. There are microfilaments, also called thin filaments in muscle made up of actin, troponin and tropomyosin. There are also microtubules made of tubulin, and there are intermediate filaments made up of a number of proteins depending on the cell type. Each is formed by polymerisation of subunits. All these filaments can be quite long (microns). Small proteins assemble to form these filaments. The types of proteins involved are different for each type, and their polymerisation processes are also quite different.

There are a wide variety of filament-associated proteins, which bind to the filaments and give them properties. For example, the associated proteins can control polymerisation and depolymerisation of the filaments, they can link filaments to membranes, organelles and extracellular material via other proteins, they can move organelles or the cell itself, and they can control the movement of motor proteins.

Most of these filaments are highly dynamic, they polymerise and depolymerise very quickly in different parts of the cell depending on the state of the cell. They also contribute to the mechanical properties of the cell, controlling cell shape, rigidity and motility. Cross-linking turns the cytoplasm from a liquid to a gel.

All three filament types are made by polymerisation of monomers. Free monomers must be in equilibrium with the polymer, but the equilibrium can be altered by other proteins that bind to either free monomers or to filaments near the site of monomer addition. The equilibrium dynamics are similar in principle for actin filaments and microtubules (they differ in detail though), but rather different for intermediate filaments. Actin filaments and microtubules have polarisation, but intermediate filaments do not.

Intermediate Filaments
These filaments are stable and durable, they are not very likely to depolymerise. Their diameter is between 8 and 12nm (intermediate in size compared with thin filaments and microtubules). They are usually prominent in cells that withstand mechanical stress, for example in the skin. The most insoluble part of the cell can be dissociated by urea. Distribution of intermediate filaments is cell-type specific, and they can be phosphorylated. Mutations lead to degenerative diseases. There are different types of intermediate filament.

Types I and II are acidic keratin and basic keratin respectively. They are found in epithelial cells (bladder, skin, etc).

Type III is found in a number of cell types, including vimentin in fibroblasts, endothelial cells and leukocytes; desmins found in desmosomes which link cells together (e.g. cardiac muscle, skeletal muscle); glial fibrillary acidic protein (GFAP) in astrocytes and other types of glia (binds prion protein) and peripherin in peripheral nerve fibres.

Type IV intermediate filaments are heavy, medium and low neurofilaments, usually found in axons. They contribute to the mechanical strength of axons. Internexin and some non-standard type IV filaments are found in lens fibres of the eye (filensin and phakinin).

Type V are lamins which have a nuclear signal sequence so they can form a filamentous support inside the inner nuclear membrane. Lamins are vital to the re-formation of the nuclear envelope after cell division. By being in the nuclear membrane, they give the nucleus its own mechanical strength.

The polymerising subunits are tissue specific (though they all have stretches of homologous structure), e.g. cytokeratins in epithelial cells, vimentin in connective tissue cells, neurofilament proteins in neurons, etc. They are usually found as a cytoplasmic network in the central region of the cell, and they provide mechanical strength to cells. In epithelial layers links at desmosomes connect intermediate filaments in adjoining cells to create strength of cell sheet.

The intermediate filament subunits come together in a parallel fashion. Filaments start forming by parallel assembly of the monomers. Dimers form staggered anti-parallel tetramers. Four tetramers form protofilaments, which then assemble into intermediate filaments. Intermediate filaments have no polarity.
**Microtubules**

These are **polymers of tubulin**. Microtubules are **hollow cylinders**. Their circumference is usually made up of 13 tubulin monomers. The microtubules have **polarity** (the two ends are not identical). One end is called the ‘**plus**’ end; the other is the ‘**minus**’ end. **Elongation occurs preferentially at the ‘plus’ end**. Tubulin dimers bind to microtubules. The dimers are made of one molecule of α-**tubulin** and one of β-**tubulin**. The β-tubulin is at the ‘**plus end**’. They are found singly or in more complex structures like cilia, flagella or centrioles.

**Tubulin** is a highly conserved protein with sub-domains which are required for subunit interactions during microtubule assembly. Tubulin has sites for **GTP binding, interaction with MAPs**, and **drug binding**. There are two types of tubulin. α-**tubulin** has a bound GTP that does not hydrolyse. β-**tubulin** has a bound GTP or GDP. The bound GTP can be **hydrolysed**, releasing Pi. GDP can be released and exchanged for GTP γ-tubulin is found in centrosomes and act as nucleating sites for microtubule assembly.

Polymerisation occurs in 2 phases.

1. **Nucleation**: the process requires tubulin, Mg$^{2+}$ and GTP and also proceeds at 37°C. This stage is relatively slow until the microtubule is initially formed. An α and a β tubulin molecule join to form a heterodimer.

2. **Elongation**: made by polymerisation of tubulin (αβ dimer, 2 x 55kDa proteins) to form a hollow filament, 25nm diameter. Around the periphery are 13 tubulin dimers, forming longitudinal, staggered 13 protofilaments.

**GTP** bound to β-tubulin is hydrolysed to produce GDP and Pi. GTP bound to α-tubulin is not. Hydrolysis stops at 4°C. Microtubules have an inherent polarity, determined by polarity of tubulin assembly.

During **microtubule assembly**, GTP binds to tubulin β-subunits and is subsequently hydrolysed to GDP. Tubulin subunits are added to GTP-capped molecules much more efficiently than to GDP-microtubules. So, if GTP-containing subunits are present at the ‘plus’ end, then the filament is relatively stable and further **polymerisation** will occur. If polymerisation is slow so that hydrolysis occurs faster than new subunit addition, GDP-containing subunits may be present at the end in which case **depolymerisation** is favoured. Replacing GTP with a non-hydrolysable analogue of GTP does not prevent polymerisation, but does prevent depolymerisation. In a healthy cell, the presence of GTP maintains microtubules.

The diagram shows the **staggered packing** of tubulins along each of the 13 protofilaments. There is fast growth by addition of αβ dimers at the plus end where there is exposed β-tubulin-GTP.

Microtubule (and actin filament) subunits contain a **nucleotide**, and for tubulin this is **GTP**. After polymerisation this hydrolyses slowly to GDP. In the case of actin filaments the nucleotide is ATP/ADP.

The switch between filament growth and depolymerisation is known as **dynamic instability**. Polymerisation / depolymerisation of microtubules depend on cellular concentrations of the microtubules, GTP, GDP, tubulin and **microtubule associated proteins** (MAPs) which affect the stability of the plus and minus-ends of microtubules. Free GDP subunits resulting from depolymerisation are converted to GTP subunits by nucleotide exchange. Since GTP hydrolysis is energetically favoured, microtubule polymerisation can do work in the cell.

In **interphase** cells, microtubules extend rapidly from the microtubule organising centre (MTOC) associated with the centrosome. The minus ends are capped by the centrosome (2 centrioles arranged at right angles to each other from which 9 triplet microtubules radiate. The plus end of the microtubules is at the periphery. **Capping** of microtubules by pericentriolar material (PCM) gives stability to microtubules even at low tubulin concentration. The ability of the spindle microtubules to be broken down by the cell during mitosis is essential for chromosomal separation.
During **mitotic spindle assembly**, some microtubules are stabilised by the proteins of the **kinetochore**. During metaphase, subunits are added to the plus end of a microtubule at the kinetochore and are removed from the minus end at the spindle pole (**microtubules maintain constant length**). At anaphase the chromatid is released from attachment to its sister at the metaphase plate and the kinetochore moves up the microtubule, removing subunits from its plus end as it goes (chromatid carried to spindle pole). Part of the chromatid movement is due to the simultaneous loss of tubulin subunits from the minus end of the microtubules at the pole. **Treadmilling** is growth at one end, and depolymerisation at the other end. Proteins attached to microtubules eventually end up at the minus end of the microtubules.

Microtubules are essential for many critical cellular processes including cell division. Their polymerisation and depolymerisation is controlled by a number of **regulatory systems** and **accessory proteins**. The microtubule system is a **target** for anti-cancer drugs like Colchicine, Vinblastine, Nocodazole, Taxol and Vincristine, and also a target for herbicides and pesticides. **Microtubule associated proteins** bind microtubules to stabilise them, cross-link them, and attach them to other cellular components like membranes, intermediate filaments and other microtubules.

Microtubules can also form bundles, as seen in flagella and cilia. **Flagella** (longer than cilia) beat to provide the force that allows sperm to swim. **Cilia** are hair-like appendages found in respiratory tract (they function to clear mucus) and in the epithelia of the oviduct (function to transport ova towards the uterus).

**Microfilaments**

**Actin formed** filaments called microfilaments and also **thin filaments** in muscle. Filaments are made by polymerisation of **G-actin** (43kDa globular protein). In most cells microfilaments are found at the periphery, underlying the cell surface. Examples include thin filaments of muscle, the core of each microvillus in a brush border, and being involved in cell surface shape changes including those underlying cell migration.

Thin filaments are very important for **cell motility**. They are found as **filopodia** at the front of moving fibroblasts and other cells. A network of actin filaments polymerise, which drives the cell forwards. **Stress fibres** are cables of actin. It is these stress fibres that are found in fibroblasts and other cells where cell adhesion is important.

Actin monomers (G-actin = Globular actin) contain ATP or ADP. **ATP is slowly hydrolysed upon polymerisation.** When this happens, there is a structural change in the various globes of the actin molecule, which makes it sticky for another actin molecule. Each repeat contains 13 molecules. Each actin filament looks like two long-pitch helices slowly winding around each other.

**Actin** (the amino acid sequence of actin) is highly conserved. Several actin-like proteins are found. These usually bind actin and co-polymerise with it. The flat F-actin structure is very much like the bacterial analogue of actin MreB. Actin is a bridge between eukaryotes and prokaryotes.

Actin exists in two forms. **G-actin** (globular actin) are actin monomers. **F-actin** (filamentous actin) are actin filaments. Actin monomers bind ATP. The ATP can be hydrolysed to form ADP-actin and Pi. There is a two step polymerisation.

1. **Nucleation**: requires ATP-actin monomers at a high concentration to form trimers of ATP-actin. Under some conditions, actin dimers are formed. This allows the formation of branching actin structures. Nucleation rate is proportional to \([\text{actin}]^3\). In motile cells, the leading edge nucleates actin polymerisation by the Arp2/3 complex and WASP family proteins.
2. **Elongation**: this is fast in the presence of ATP-actin. ATP-actin monomers are added to actin trimers to form new filaments and at the ‘barbed’ end of actin filaments to elongate existing actin filaments. All subunits have identical polarity. The filament is helical. Polymerisation at the ‘barbed’ end is 10x faster than at the ‘pointed’ end. Elongation at the leading edge may push the membrane forward.

The description of the herring-bone structure is seen when actin filaments are ‘decorated’ with myosin molecules. This is an indication of polarity of the actin filaments. The rate of growth at the barbed end is about 10x faster than at the pointed end.
The ATP in F-actin hydrolyses so that the bulk of actin filaments contain ADP-actins. At low concentrations of ATP-actin monomers, actin monomers at the ‘barbed’ ends of F-actin will end up with ADP-actins. These will dissociate from F-actin, causing shortening, unless stabilised by ligands. ADP in ADP-actin monomers can be exchanged for ATP.

There are many examples of actin-binding proteins in muscle. There are many classes of myosin motors, there is troponymosin (part of thin filaments in smooth and striated muscle involved in regulation), troponin (confers Ca-regulation of contraction to thin filaments in skeletal muscle), caldesmon (smooth muscle regulation of contraction), calponin (smooth muscle regulation) and α-actinin (cross-linking protein).

There are also many examples of actin-binding proteins in the cytoplasm. There is α-actinin (cross-linking protein), gelsolin (capping, nucleating and severity activity, calcium activated capping and severing, also bundles actin filaments), villin (nucleates, severs and caps actin filament), profilin (binds actin monomers and provides pool for actin elongation at barbed end), cofilin (regulated by phosphorylation, binds to G- and F-actin, increases filament turnover 20-30x), fimbrin (α-actinin like domains, can bundle actin filaments), vimentin (found in intermediate filaments, maintains myofibril alignment in striated muscle), vinculin (actin cross-linking and bundling), ezrin, radixin, and moesin (regulation by phosphoinositide lipids, active in unfolded tail conformation).

Molecular motors are proteins which have evolved to move cells or parts of cells. The movement requires energy, so molecular motors are usually ATPases (hydrolyse MgATP to MgADP and inorganic phosphate). Sometimes they harness ion gradients (bacterial flagellae). Kinesin moves vesicles along microtubules. In the absence of ATP you get rigor mortis.

Learning Objectives for this lecture:
- Review previous knowledge of the cytoskeleton and the three filament systems which make it up.
- Describe the assembly and organisation of subunits in intermediate filaments.
- Explain why these filaments do not show a polarity and how the same class of filament can be made from different subunit molecules in different cell types.
- Define the role of intermediate filaments, their size and localisation.
- Describe how the structure of microtubules results from polymerisation of subunits consisting of α,β-tubulin dimers.
- Describe the polymerisation dynamics of microtubules in terms of preferred (+) and non-preferred (-) ends and GTP hydrolysis.
Invasion - Regulation of Cell Migration
by Dr Vania Braga

How does detachment from the primary tumour and migration occur? What are the molecular mechanisms that regulate motility?

There is a complex interplay between cell-cell adhesion, and cell attachment to the ECM. These determine cell shape, and require a dynamic cytoskeleton to be maintained. Any perturbation of these will change the phenotype of the cell from a more sessile cell to a more motile cell.

A prime example is during tumour progression. In epithelial tissue (which is involved in 80-90% of cancers), there is a layer of cells tightly attached to each other lying on a basement membrane. Underneath this is a stroma with blood vessels and supportive tissues.

If there are genetic alterations leading to hyperproliferation in some cells, these cells will de-differentiate and lose their function as an epithelial cell. They disassemble cell-cell contacts, and lose polarity.

Subsequently, the cells invade, which requires cleavage of ECM proteins and increased motility. There is then metastasis.

Under the microscope, metastatic cells migrate much faster than normal cells. They don’t attach to neighbours, and they move everywhere in every direction. They move all over each other and don’t stop when they should stop by contact inhibition.

An example of a study is inoculating tumour cells in a mouse. The tumour cells form a primary tumour, and you can stick in a needle with growth factors to stimulate the cells to migrate towards it. Once you extract these cells you can compare the expression profile of invasive cells compared to those in the primary tumour by measuring mRNA levels. You’ll find there is up-regulation in genes involved in cytoskeleton regulation and motility machinery.

Cell Movement
Stimuli for cells to move include organogenesis and morphogenesis, wounding, growth factors / chemoattractants, and de-differentiation (tumours). The cells change their morphology so they become polar to have directionality. These changes are co-ordinated by the cytoskeleton. Normal cells stop when they find their neighbouring cells (contact inhibition of motility). The cells develop specialised structures to allow movement (e.g. focal adhesions, lamellae, filopodia).

A key issue in motility is that the cells need to attach somewhere. Usually there is attachment to the substratum (ECM proteins). There are points in the cell where it is very close to the substratum, and these are focal adhesions. Stress fibres (bundles of actin) are cables that attach to focal adhesions. Transmembrane proteins allow attachment to the ECM - the best example is integrins. On the intracellular side, they interact with various cytoskeletal proteins to form a plaque. The plaque mediated the attachment.

Filopodia are finger like protrusions rich in actin filaments. Lamellipodia are sheet like protrusions rich in actin filaments. These attach to the substratum. When they move back, the structures are called “ruffles”.

An analogy to cell movement is wall climbing.
During cell movement, control is needed within a cell to co-ordinate what is happening in different parts of the cell. Control is also needed to regulate adhesion and release of the cell-ECM receptors, and control is needed from the outside to respond to external influences. This requires sensors and directionality.

Hapoptatic motility is movement with no direction. Chemotactic motility is where the cell senses a stimulus and goes towards it. In both cases, cell movement involves changing cell shape.

Cells regulate shape changes by regulating the actin cytoskeleton. Actin can be found as small soluble subunits (G-actin), and can polymerise into large filamentous polymers (F-actin). If there is a signal like a nutrient source at one end of the cell, the F-actin at the other end will disassemble and the subunits will diffuse to the side with the signal. There is reassembly of the subunits at the new site and this facilitates movement.

A migrating fibroblast cell has actin filaments in filopodia, the cell cortex and in stress fibres. In the filopodia they form tight parallel bundles; in the cortex they form a gel-like network to provide support to the plasma membrane; and in stress fibres they form contractile bundles.

Different actin binding proteins can do specific functions to drive specific cell structures. The actin cycles between being a monomer and in a filament. The monomer in solution polymerises very quickly, because the lowest energetic state is as a filament, but you can't just have loads of filaments in the cell. The solution to this is to have sequestering proteins, which store the G-actin monomers until they are required. Motor proteins provide contraction, and other proteins bind to stabilise the filaments. Capping proteins are stop signals which prevent further growth. Severing proteins are like scissors.

**Nucleation** is the limiting step in actin dynamics - the formation of trimers to initiate polymerisation. This is a difficult step, and so there are Arp proteins (actin-related proteins) which are similar to actin which form a complex able to form a trimer of actin. Once the trimer is done, then the filament can elongate.

**Elongation** brings monomers (usually sequestered) to the filament. Profilin binds to the monomers and helps to elongate the filament. Thymosin is a sequestering protein which prevents polymerisation. There is a tug of war between these two proteins to regulate filament growth, as profilin competes with thymosin for binding to actin monomers and promote assembly.

**Branching** is regulated by the Arp complexes. Branches are usually at around a 70° angle, and they work their way upwards.

**Capping** prevents further growth of the filament. Capping proteins include Cap Z, Gelsolin, Fragmin and Severin at the plus end. At the minus end proteins include Tropomodulin and Arp complex.

**Cross-linking** and **bundling** are of two types. One type is actin filaments and α-actinin, which forms contractile bundling, with a loose packing allowing myosin-II to enter the bundle. The other type is actin filaments and fimbrin, which forms parallel bundling, with tight packing that prevents myosin-II from entering the bundle.

**Severing** is a scissor like activity. In an unsevered population, actin filaments grow and shrink relatively slowly. In a severed population, actin filaments grow and shrink more rapidly. Severing proteins include Gelsolin, ADF/Cofilin, Fragmin and Severin. The gel-sol transition is one of the consequences of severing. The chicken-wire network of filaments holding together the cell is broken so that filopodia etc can form. The gel is a rigid structure, and the sol allows the membrane to flow.

**Membrane connections** allow the cells to sense surfaces. They involve a transmembrane protein and a plaque on the inside with the cytoskeletal attachment. Connections to the transmembrane proteins are mediated by
cell-cell and cell-ECM receptors. There is nucleation, elongation, cross-linking and bundling, with the recruitment of signalling complexes. Proteins involved include Talin, α-catenin, Spectrin, Ezrin, Radixin and Moesin.

During cell movement there is participation of different actin activities during cell movement like disassembly, nucleation, branching, severing, capping and bundling.

Lamellae protrusion involves polymerisation, disassembly, branching and capping. During protrusion there is net filament assembly at the leading edge, and net filament disassembly behind the leading edge. Filopodia form with actin polymerisation, bundling and cross-linking to form the tight parallel bundles.

Signalling mechanisms that regulate the actin cytoskeleton include ion flux changes (i.e. intracellular calcium), control by phosphoinositide signalling and signalling cascades via small GTPases.

Actin filaments can be controlled by Ca\(^{2+}\) because for example Gelsolin severing is Ca\(^{2+}\) dependent, as this exposes the fast depolymerising end. Also cross-link binding is decreased at high Ca\(^{2+}\) levels.

The actin cytoskeleton can also be controlled by small G proteins. The Rho subfamily of small GTPases belongs to the Ras superfamily (Rac, Rho, Cdc42, etc). They participate in a variety of cytoskeletal processes. These proteins are activated by receptor tyrosine kinase, adhesion receptors and signal transduction pathways. Expression levels are up-regulated in different human tumours.

Filopodia are activated by Cdc42, lamellae are activated by Rac, and stress fibres are activated by Rho.

It is important to learn about cytoskeletal proteins because various diseases like high blood pressure, Wiskott-Aldrich syndrome, epidermolysis bulosa, bullous pemphigoid etc are caused by deregulation of the actin cytoskeleton.

**Learning Objectives for this lecture:**
- Describe the cytoskeletal processes occurring during cell locomotion.
- Explain the different actin binding proteins and the activities in which they participate to remodel actin filaments.
- Describe the role of phosphorylation and other second messengers as control mechanisms for cytoskeletal components.
- Describe the role of small G proteins of the Rho family in controlling organisation of the actin cytoskeleton.
- Explain why the onset of metastasis is a critical stage in the development of a cancer, and give examples of the changes at the cellular and molecular levels which are necessary for it to occur.
**Biological Basis of Cancer Therapy**
by Professor Justin Stebbing

The hallmarks of cancer cells is that they keep growing, keep dividing, they invade and spread. Classical anti-cancer agents produced before the last decade either target DNA synthesis or the mitotic spindle. The modern targeted drugs tend to target cell surface receptors.

Cytotoxic Discovery by Mistake
Chemotherapy has been discovered by mistake, by luck and by trial and error. Mustard gas was first used by the Germans in 1917. It was noticed that the survivors of mustard gas had conjunctivitis, sores on their hands, blistering and hair loss. Four people died the next day. Mustard gas is a very simple compound. In the Second World War, mustard gas wasn’t used, but the Americans were scared that the Germans would use it. On the U.S.S. John Harvey there was a big bomb which contained 100 tonnes of mustard gas. The Germans dropped a bomb on it, and the mustard gas bomb blew up. A very clever doctor on board noticed that at day 5 the white cells on the people exposed to mustard gas stopped increasing in number, in fact they started decreasing in number. He thought there was something in mustard gas that stopped cells dividing. In 1942, nitrogen mustard was given to a mouse with lymphoma, and this led to tumour regression. In 1944 the first patient with non-Hodgkin’s lymphoma was treated with nitrogen mustard, and the tumours regressed and he lived 2 months before dying of marrow failure.

Drug Discovery by Luck
In 1965 at the University of Michigan, a physicist called Barnett Rosenberg was studying the effects of electric currents on E.coli using platinum electrodes in a water bath. The E.coli stopped dividing but not growing, leading to long bacteria up to 300 times longer than normal. Cisplatin was the product from the platinum electrodes that was responsible for this cease of cell division.

Drug Discovery by Trial and Error
In 1971 President Nixon declared the ‘War on Cancer’, where he decided to screen every single compound under the kitchen sink etc for anti-cancer properties. They actually came up with some major classes of chemotherapy still used today. Classes of chemotherapy from nature include Vinca alkaloids, Taxanes, Camptothecans, Anthracyclines, Bleomycin, Epipodophyllotoxins and Actinomycin.

Chemotherapy
This works by targeting cells that divide too fast. Chemotherapy works in two ways - by targeting the formation of DNA bases, by targeting the DNA to inhibit synthesis or by targeting the mitotic spindle. These are the major differentiators between modern targeted therapies which target cell surface receptors. The broad classes to know for the exams are:

1. **Anti-metabolites**: inhibit base synthesis
2. **Alkylating agents**: bind to DNA bases
3. **Intercalating agents**: disrupt double helix
4. **Topoisomerase inhibitors**: disrupt DNA supercoiling
5. **Spindle cell poisons**: inhibit mitotic spindle.

Anti-metabolites inhibit base synthesis. There are either false bases like purine analogues or pyrimidine analogues, or there is the inhibition of the enzymes that synthesise DNA bases using dihydrofolate reductase inhibitors / thymidylate synthetase inhibitors. Common purine analogues include 6 Mercapto-purine and 6 Thio-guanine, and common pyrimidine analogues include 5 Fluorouracil and Gemcitabine.

Anti-metabolites were first noticed in the 1940s when there were pregnant women who were bed bound with large red blood cells. When they had Marmite, they ran out of the hospitals because their folate deficiency had been corrected. On giving folic acid to leukaemia patients, they died very quickly. Aminopterin was the first anti-folate synthesised, and in 1947, sixteen children with acute leukaemia were treated with Aminopterin and ten achieved temporary remission. Nowadays because of these drugs, most paediatric leukaemias are curable.

**Metotrexate** is the daughter of Aminopterin. It is all that is needed now for placental carcinomas. They provided the first ever cures (durable remissions) with chemotherapy alone reported with Methotrexate for choriocarcinoma in 1963.
Drugs that disrupt DNA double helix and interfere with DNA synthesis are Alkylating agents (bind to DNA bases), Intercalating agents (disrupt double helix) and Topoisomerase inhibitors (disrupt DNA supercoiling).

Alkylating agents transfer an alkyl group to either N7 of the purines or to the O6 of guanine. As a result of this alkylation you get interstrand linkages.

Cisplatin (from platinum electrodes) is an intercalating agent. These disrupt the double helix. You could be asked in the exams to describe how anti-DNA agents work in cancer chemotherapy, or describe something about where they come from. But Professor Stebbing didn’t elaborate on intercalating agents, so that’s that.

Topoisomerase inhibitors like Etoposide are from a podophyllotoxin from the roots of Podophyllum emodi, a cousin of the mayapple. In cancer there are too many cells dividing. Some classes of chemotherapeutics attack the mitotic spindle. A lot of drugs bind to specific proteins like microtubules, but this is not referred to as “targeted therapies”. This phrase is used to describe molecules that bind to receptors on the surface of the cells.

Taxanes like Paclitaxel are from Pacific Yew bark, and Docetaxel is from European Yew needles. Taxanes bind β-tubulin, and inhibit tubulin depolymerisation inhibiting mitotic spindle formation.

Vinca alkaloids prevent β-tubulin polymerisation to inhibit spindle formation. These include Vincristine, Vinblastine, Vindesine, and VInorelbine.

As a general rule, chemotherapeutics are cell cycle specific. The majority act in the S phase of the cell cycle when the cells are making DNA.

Drugs often may not work for a number of reasons.

The first is multi-drug resistance. Most drugs lead to up-regulation of the APC gene, which encodes the P-glycoprotein pump also called MDR-1. Once this is up-regulated, it can pump a massive list of drugs out of the cell. This is because it pumps out toxins including most cytotoxics. Methotrexate, Cisplatin, Vinca Alkaloids, Taxanes, etc can all lead to the up-regulation of this pump. But once it is up-regulated, it can pump all the other drugs out of the cell.

Other reasons are usually cytotoxic drug specific resistance. Drug specific mechanisms often involve reduced drug uptake, reduced drug activation, increased detoxification, altered target levels and repair of drug induced damage.

Methotrexate inhibits dihydrofolate reductase used to make bases. It is an anti-folate, anti-Marmite, and it inhibits pyrimidine synthesis. But in some individuals there is massive amplification of the dihydrofolate reductase gene, and those individuals will become resistant to Methotrexate.

Chemotherapy has predictable side effects. For fast growing normal cells, it inhibits cell division and cell cycle specific drugs affect bone marrow, GI tract epithelium, hair and nails, and spermatogonia. For slow growing normal cells, it introduces DNA mutations, cell cycle independent (Alkylating agents) secondary tumours. The commonest side effects ranked by severity by patients are nausea, tiredness, hair loss, concern about effect on friends and family, and vomiting.

Toxicities can be classified too. Immediate toxicities (hours after) include nausea and vomiting, anaphylaxis and extravasation (tissue damage). Delayed toxicities (days/weeks) include myelosuppression (low blood cells), stomatitis (sore mouth), alopecia, and neuropathy. Late toxicities (years) include infertility and secondary tumours. A lot of drugs now can cause heart failure too.

Radiotherapy
Radium was first used in 1901 by a Paris dermatologist who applied a radium mixture to a patient’s skin cancer. In the 1940s, linear accelerators were developed. Radiotherapy is usually given by CT scanning the patient and using lasers to define the target (cancer). The inverse square law is used when giving radiotherapy to patients.

Radiotherapy works in 4 steps. Firstly, an electron from the radiotherapy displaces an orbiting electron. There is then ionisation of a water molecule, and a hydroxyl radical is formed. This radical then damages DNA.

1 Gray is the dose absorbed when 1 joule is deposited in 1kg of tissue. Each Gray per cell causes 10,000 damaged DNA bases, 1000 damaged deoxyribose sugars, 1000 single strand breaks, 40 double strand breaks, 150 DNA-protein cross links, and 30 DNA-DNA cross links. A typical radiotherapy course for breast cancer may be about 30 Grays in 30 fractions, for spinal cord may be 25 Grays in 5 fractions. A fraction is an amount per day.

**Hiroshima** was bombed on the 6th August 1945 at 8:15am. The effects of nuclear bomb irradiation vary with time. Within 1 or 2 hours, the effect is radiation sickness (acute vomiting). Within 2 to 14 days the effects include a denuded intestinal epithelium (diarrhoea, GI haemorrhage and septicaemia). Within 14-21 days there is myelosuppression and pancytopenia (neutropenic sepsis and haemorrhage). In 3 to 10 years people may suffer from acute myeloid leukaemia. Over 40 years people may develop solid tumours.

**New Targets, New Drugs**

There are six steps to cancer:
1. Self sufficiency in growth stimuli
2. Insensitivity to inhibitory stimuli
3. Evasion of apoptosis
4. Immortalisation
5. Neoangiogenesis
6. Invasion and metastasis

The first thing is autonomous growth signals. The epidermal growth factor receptor is a typical cell surface tyrosine kinase receptor. Taxanes target and bind to these receptors (to β-tubulin). By convention the targeting agents bind to cell surface receptors. Nibs are small peptide tyrosine kinase inhibitors. Mabs are monoclonal antibodies. Drugs include Iressa, Tarceva, Erbitux, Gefitinib, Elotinib and Cetuximab.

To make a monoclonal antibody, you combine tumour cells with B cells from the spleen of immunised mice. The key step in making the antibodies suitable for modern oncology is their humanisation. You take the antibodies and cleave off the Fab fragment and attach them to a human antibody. Humanisation = zumab.

In the exam, you may be asked to give an example of a targeted therapy. The best example is Herceptin. Cancer is a heterogenous disease. Breast cancer is made up of about 30 different gross types clinically. One of the types over-expresses a gene called Her2 (human epidermal growth factor receptor 2). When it is over-expressed in the 15% of breast cancers it is expressed in, it is expressed 250 times the normal amount. This comes from an increased gene copy number, increased transcription and increased protein synthesis. It is a tyrosine kinase receptor, so it phosphorylates intracellular proteins like MAPK etc, and it represents the “on” signal in the cell.

**Herceptin** is an amazing drug, as 1 year of it increases absolute survival by 8%. Say there is a patient with a 75% chance of cure from surgery alone, but then Herceptin increases this by the 8% of the 25% who would have died. The cost of 1 year adjuvant Herceptin (Trastuzumab) is £21,000. There are some risks of cardiac events. Herceptin is the best example of a targeted drug. Methotrexate is the best example of a traditional drug.

The second thing is ignoring cell cycle arrest points. The problem with CDK inhibitors is that every cell needs to divide, so the side effects are likely to be broad, unlike anti-Her2 therapy which just targets cells with over-expressed Her2 (breast cancer cells).
Bcl-2 prevents apoptosis and is over produced by cancer cells. Turning off Bcl-2 production should lead to programmed cell death. Antisense drugs like oligonucleotide can be used in this situation.

Fibroblasts can divide 120 times (Hayflick phenomenon). You can take fibroblasts, put them in media deprived of nutrients, and keep them like that for years. If you add back in the nutrients, they remember how many divisions they have had and they can then keep dividing only up to 120 times. Cancer cells avoid getting older, unlike fibroblasts, by producing telomerase (hTERT) that restores the telomere ends.

Neoangiogenesis inhibitors include Bevacizumab, Avastin, Monoclonal antibody, and VEGF. They straighten out blood vessels in tumours, and work best with chemotherpay.

Primary tumours invade local tissues and release circulatory tumour cells to develop metastases. To invade, there are 26 human matrix metalloproteinases, and there are inhibitors being developed against these.

Phase I trials in drug development aim to determine the toxicity and dose scheduling of the drug. They are tried on few patients, for whom no known alternative therapy is available. The endpoints will indicate activity in humans, the maximum tolerated dose, pharmacokinetics and side effects related to different doses.

Phase II trials aim to identify promising tumour types. The drugs are tried on more patients than phase I trials treated at the dose and schedules determined in phase I. The endpoints indicate activity in human tumour types and side effects.

Phase III trials aim to compare the efficacy and toxicity with standard therapy. The drug is tried on a large number of patients randomised and controlled usually multicentre. The endpoints show a comparison with standard therapy and side effects.

The RECIST criteria for a response are:
- **Complete response**: disappearance of all known disease
- **Partial response**: >50% reduction in measurable lesions and no new ones
- **Stable disease**: lesions unchanged (<50% smaller or <25% larger)
- **Progressive disease**: new lesions or measurable lesions >25% larger

2% of the population of the UK (1.2 million people) are alive having received a diagnosis of cancer. These are mainly breast cancer patients (180,000). In terms of childhood cancer survivors there are 1 in 600 children (often inherited risk). 7 in 10 are cured. In the UK there are 55,000 young adults who are cancer survivors.

Late side effects are of surgery, radiotherapy and chemotherapy. There may also be psychological consequences. For example, Lazarus syndrome is difficulty with returning to normal life, Damocles syndrome is fear of recurrence and terror of minor symptoms, Survivor syndrome is guilt about surviving when others have died.

**Learning Objectives for this lecture:**
- Describe the main chemotherapeutic and radiotherapeutic approaches to treating cancer.
- Explain why many cancer treatments cause side effects such as nausea, hair loss, anaemia and immunosuppression, and indicate the approaches which have been tried to minimise these.
- Explain the rationale for the newer drugs in cancer therapy.
- Discuss the prospects for new therapies based on the biology of cancer development.
Cellular Pathology of Cancer
by Dr Marjorie Walker

Metaplasia is a reversible change in which one adult cell type (epithelial or connective tissue) is replaced by another adult cell type. This is an adaptive process.

Dysplasia is an abnormal pattern of growth in which some of the histological features of malignancy are present. This is a pre-invasive change, where there is an intact basal membrane. There is loss of architectural orientation, loss in uniformity of individual cells, variability in size and shape, nuclei are hyperchromatic and enlarged, and mitotic figures are abundant and in places where not usually found. Dysplasia is common in the cervix (HPV infection), bronchi (smoking), colon (ulcerative colitis), larynx (smoking), stomach (pernicious anaemia), and oesophagus (Barrett’s metaplasia).

Neoplasia is an abnormal autonomous proliferation of cells unresponsive to normal growth control mechanisms which even persists in proliferating when the original stimulus is finished.

Benign tumours do not invade, do not metastasise, are encapsulated, are usually well differentiated, are slowly growing and show normal mitoses. Benign tumours are not often fatal unless they are in a dangerous place like the meninges, pituitary gland, or unless they secrete something dangerous like an insulinoma. They may also be fatal if they get infected (e.g. bladder), if they bleed (e.g. gastric muscle), if they rupture (e.g. cysts of ovary, liver adenoma), or if they become torted or twisted they may infarct (e.g. ovarian cyst).

Malignant tumours invade surrounding tissues and spread to distant sites. They have no capsule. They can be well to poorly differentiated, and are rapidly growing. They show abnormal mitoses. A metastasis is a discontinuous growing colony of tumour cells, at some distance from the primary cancer. Common patterns of metastatic spread depend on the lymphatic and vascular drainage of the primary site. From the colon, the portal vein drains to the liver, so the most common site for secondary metastasis is the liver. From the breasts, the lungs are the most common secondary site. Lymph node involvement usually has a worse prognosis. A staging system used is the Dukes system. In the colon for example, Dukes A - there is a 90% five year survival rate, Dukes C (penetrated through the bowel wall) - 30% five year survival.

Carcinoma = a malignant tumour derived from epithelium. Carcinomas may be squamous, adenocarcinoma, transitional cell carcinoma, basal cell carcinoma, and various qualifying names.

Leukaemia & Lymphoma = tumours of white blood cells. Leukaemia is a malignant tumour of primitive bone marrow derived cells which circulate in the blood stream. Lymphoma is a malignant tumour of lymphocytes proliferating (usually) in lymph nodes.

Teratoma = a tumour derived from germ cells, which has the potential to develop into tumours of all three germ cell layers - ectoderm, mesoderm and endoderm. They are common in the gonads, but occur in midline situations too. They can occur in the pituitary, pineal gland, mediastinum, and sacro-coccygeal areas too. Gonadal teratomas are all malignant in males, whereas in females most are benign.

Hamartoma = localised overgrowth of cells and tissues native to the organ. Cells are mature but architecturally abnormal. They are common in children and should stop growing when they do. Examples include haemangiomas, bronchial hamartomas, and Peutz-Jegher polyps in the gut.

Benign epithelial tumours on surface epithelium = papilloma often on skin, bladder, colon, etc. On glandular epithelium = adenoma often on glands, maybe secretory of mucin, thyroid colloid, etc. They occur in the stomach, thyroid, breast, colon, kidney, pituitary, pancreas and parathyroid glands.

Sarcoma = a malignant tumour derived from connective tissue or mesenchymal cells. In fat this is a liposarcoma. In bone this is an osteosarcoma. In cartilage this is a chondrosarcoma. In striated muscle this is rhabdomyosarcoma and in smooth muscle this is leiomyosarcoma. In nerve sheaths this is a malignant peripheral nerve sheath tumour.
There are criteria for assessing differentiation of a malignant tumour. If evidence of normal function is still present (e.g. production of keratin, bile, mucin, hormones, etc) it is unlikely to be high grade. If there is no evidence of this, then the tumour is anaplastic. Mitoses are important, particularly when abnormal. There are various grading systems, e.g. for breast, prostate, colon, etc. If there is no differentiation, then it is an anaplastic carcinoma.

**TNM grading** is often used. The grade of a tumour describes its degree of differentiation. The stage of a tumour describes how far it has spread. Tumours of higher grade (i.e. more poorly differentiated) tend to be of higher stage. Overall, stage is more important than grade in determining prognosis. Different tumours have different staging systems, for example the prostate.

The Tumour, Node, Metastasis (TNM) system can be applied, and individualised, to tumours in all sites.

**Learning Objectives for this lecture:**
- Define the words metaplasia, dysplasia, neoplasia, tumour, malignancy, hamartoma, carcinoma, sarcoma, teratoma, lymphoma, leukaemia, carcinogen, and metastasis.
- Explain the principles underlying the nomenclature of tumours.
- List four features which distinguish benign from malignant tumours and explain how they are of use in making that distinction.
- Describe five morphological features which allow assessment of the differentiation of a tumour.
Colorectal Cancer by Dr Michael Osborn

Colorectal cancer is a major cancer in developed countries. It is the 4th most common cancer overall, and is the 2nd leading cause of cancer death overall, behind lung cancer. There are both environmental (diet) and genetic factors in aetiology.

The colon is involved in the extraction of water from faeces, and this contributes to electrolyte balance. It can also act as a faecal reservoir if need be, and it is also involved in bacterial digestion for vitamins like vitamin B and K.

Between 2 and 5 million cells die per minute in the colon, and proliferation renders the cells vulnerable. APC mutations prevent cell loss. Normally we have protective mechanisms to eliminate genetically defective cells by natural loss, DNA monitors, and repair enzymes.

A polyp is any projection from a mucosal surface into a hollow viscus, and may be hyperplastic, neoplastic, inflammatory, hamartomatous, etc. An adenoma is a benign neoplasm of the mucosal epithelial cells.

Colonic polyps may be metaplastic/hyperplastic, adenomas, juvenile, Peutz Jeghers, lipomas, or others (essentially any circumscribed intramucosal lesions).

Hyperplastic polyps are very common, and are usually less than 0.5cm in size. About 90% of all large intestine polyps are hyperplastic. There are often multiple polyps, and there is no malignant potential. 15% have a k-ras mutation.

Colonic adenomas are of various types. 90% are tubular, and 10% are tubulovillous. Adenomas may be villous, flat or serrated.

- Tubular adenomas are columnar cells with nuclear enlargement, elongation, multi-layering and have a loss of polarity. They have increased proliferative activity with reduced differentiation. Their architecture is complex and disorganised.
- Villous adenomas are mucinous cells with nuclear enlargement, elongation, multi-layering and loss of polarity. They have exophytic, frond-like extensions, and rarely may have hypersecretory function and result in excess mucus discharge and hypokalaemia.

Dysplasia literally means ‘bad growth’. A colonic dysplasia is an abnormal growth of cells with some features of cancer, although this requires subjective analysis. They may be indefinite, low grade, or high grade.

Adenomatous Polyposis Coli (APC/FAP) is a disease caused by a 5q21 gene mutation. The site of mutation determines the clinical variants (classic attenuated, Gardner, Turcot, etc). Many patients have prophylactic colectomy.

25% of adults have colonic adenomas at age 50. 5% of these become cancers if left. Large polyps have a higher risk than small ones. Cancers stay at a curable stage for about 2 years.

Most colonic carcinomas arise from adenomas. There is a residual adenoma in 10 to 30% of CRCs. Adenomas and carcinomas have a similar distribution, and adenomas usually precede cancer by 15 years. Endoscopic removal of polyps decreases the incidence of subsequent CRC.

Genetic pathways include the Adenoma Carcinoma Sequence (APC, K-ras, Smads, p53, telomerase activation) and Microsatellite instability. Microsatellites are repeat...
sequences prone to misalignment. Some microsatellites are in coding sequences of genes which inhibit growth or apoptosis e.g. TGFβR11. Mismatch repair genes (MSH2, MLH1 and four others) are recessive genes requiring 2 hits.

The two main pathways are:
- **FAP**: inactivation of the APC tumour suppressor genes
- **HNPCC**: microsatellite instability.

The **Adenoma-Carcinoma sequence**:

There are 35,000 cases of colonic carcinoma per year in the UK, and they form 10% of cancer related deaths (16,000 per year). The age ranges from 50 to 80, with sporadic rare cases in people under 30. Colonic carcinoma is high in the USA, Eastern Europe and Australia. Rates are particularly low in Japan, Mexico and Africa. **Dietary factors** are very important = high fat, low fibre, high red meat, refined carbohydrates.

**Environmental Factors - Diet**

Food contains 5 to 10,000 bioactive chemicals, and food also contains carcinogens as well as anti-cancer agents. Heat modifies chemicals further, and bacteria can modify food residues. HCAs (heterocyclic amines) are generated when meat is cooked at high temperatures. PhIP oxidation \( \rightarrow \) N-OH-PhIP + Doxyguanosine \( \rightarrow \) Mutagenesis.

Dietary deficiencies are also significant in colorectal cancer. Folates are important, as a co-enzyme is needed for nucleotide synthesis and DNA methylation. MTHFR deficiency leads to disruption in DNA synthesis causing DNA instability (strand breaks and uracil incorporation) which leads to mutations. Decreased methionine synthesis leads to genomic hypomethylation and focal hypermethylation, which leads to gene activation and silencing.

Anti-cancer food elements include vitamin C (ROS scavenger), vitamin E (ROS scavenger), isothiocyanates (cruciferous vegetables), and polyphenols found in green tea and fruit juice (activate MAPK, which regulates phase 2 detoxifying enzymes as well as other genes like glut-S-transferase and also reduces DNA oxidation). Other health food mechanisms include garlic associated apoptosis (Ajoene, allicin), and green tea (EGCG-induced telomerase activity).

**Clinical Presentation of Colorectal Carcinoma**

There is usually with a change in bowel habit, bleeding per rectum and unexplained iron deficiency anaemia. There may also be mucus PR, bloating, cramps (colic) and constitutional weight loss and fatigue. Patients rationalise these symptoms as ‘getting old, piles or irritable bowel’, and so do doctors.

Small carcinomas may be present with larger polypoid adenomas, pedunculated or sessile. 22% are in the caecum and ascending colon, 11% are found in the transverse colon, 6% in the descending colon and 55% in the rectosigmoid.
**Duke’s Staging System**

**Grading** is the *proportion* of gland differentiation relative to solid areas or nests and cords of cells without lumina.

About 10% are well differentiated, 70% moderately and 20% poorly differentiated.

**Dukes Classification:**
- Dukes A = growth limited to wall, nodes negative
- Dukes B = growth beyond musc propria, nodes negative
- Dukes C1 = nodes positive, apical lymph nodes negative
- Dukes C2 = apical lymph nodes positive

<table>
<thead>
<tr>
<th>Clinical features</th>
<th>Effect on prognosis</th>
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<tr>
<td>Rectal bleeding as presenting symptom</td>
<td>Improved prognosis</td>
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<td>Bowel obstruction/perforation</td>
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<td>Tumor location</td>
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<td>Preoperative serum CEA</td>
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<td>Distant metastases</td>
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<tr>
<th>Pathologic features</th>
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<tr>
<td>Depth of bowel wall penetration</td>
<td>Increased penetration diminishes prognosis</td>
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<tr>
<td>Number of regional lymph nodes involved</td>
<td>1-4 nodes better than &gt;4 nodes</td>
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<tr>
<td>Degree of differentiation</td>
<td>Well &gt; poorly differentiated</td>
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<td>Mucinous (colloid) or signet ring cell</td>
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<tr>
<td>Venous Invasion</td>
<td>Diminished prognosis</td>
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<tr>
<td>Lymphatic Invasion</td>
<td>Diminished prognosis</td>
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<tr>
<td>Perineural Invasion</td>
<td>Diminished prognosis</td>
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<tr>
<td>Local inflammation and immunologic reaction</td>
<td>Improved prognosis</td>
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The best *treatment* option for colorectal cancer is *surgery*, and alongside treatment with 5FU, Leucovorin, Metastatectomy, Chemotherapy and Palliative RT.

**Screening Programmes**

**Screening** for high risk colon cancer includes picking out patients with previous adenoma, 1st degree relatives affected before the age of 45, 2 affected first degree relatives, evidence of dominant familial cancer trait, ulcerative colitis and Crohn’s disease, and hereditable cancer families.

Screening is the practice of *investigating* apparently healthy individuals with the object of detecting unrecognised disease or people with an exceptionally high risk of developing disease, and of *intervening* in ways that will prevent the occurrence of disease or improve the prognosis when it develops.

The criteria for a screening programme include the *importance of the disease* (condition should be important in respect to the seriousness and/or frequency), and the *natural history of the disease must be known* (in order to identify where screening can take place and to enable the effects of any intervention to be assessed). Tests should be *simple* and *acceptable* to the patient, should be *sensitive* and *selective*. The screening population should have *equal access* to the screening programme, and it should be *cost effective*. Most people aged 60-69 are screened every 2 years, and positives are referred for colonoscopy.

**Learning Objectives from this lecture:**
- Describe the different modes of clinical presentation of colorectal carcinoma.
- List the principles of the adenoma-carcinoma sequence.
- Define the Duke’s and TNM staging systems.
- Develop an understanding of molecular pathogenesis of colorectal carcinoma.
- Describe the major pathological features which are associated with aggressive malignant behaviour of colorectal carcinoma.
Skin Cancer
by Professor Tony Chu

Skin cancer is the commonest cancer to affect man. The main aetiological agent is sunlight. Additional factors that may be important include ionising radiation, viruses and tissue scarring. The three commonest skin cancers in ascending order of incidence are basal cell carcinoma, squamous cell carcinoma and melanoma. Aggressiveness of the tumour increases with decreasing incidence.

Basal cell carcinoma is generally a tumour of the elderly but with more recreational sun exposure it is seen in patients from the teens. These tumours arise from pluripotent stem cells within the epidermis and the histological subtype is controlled to an extent by the underlying stroma. They are commonest in certain sun exposed areas - face, ears, neck, and are frequently seen on the back, chest and legs but rarely on the backs of hands or arms. They are locally destructive but generally not metastatic. Regional lymph node metastases are only seen with large and long standing tumours.

Squamous cell carcinomas are tumours of the elderly. These tumours develop from basal keratinocytes, and are commonest in sun exposed areas. Tumours are metastatic spreading to regional lymph nodes then to solid organs, particularly the lungs. Tumours may arise from precursor lesions like solar keratoses and Bowen’s disease.

Melanoma is tumour of the young, but may arise at any age. It is a tumour arising from epidermal melanocytes or rarely dermal melanocytes. They are associated with intermittent intense sun exposure, and with sunburn before the age of 15 years. They are actually unusual in sun exposed areas, and are highly metastatic spreading to regional lymph nodes then solid organs like the lungs, liver and brain.

Major risk factors for melanoma are the presence of dysplastic naevae, and a family history. Other risk factors include being in higher socio-economic groups, arsenic exposure in farmers, airline pilots have 3x risk due to ionising radiation, correlates with reduced cost of travel to sunny locations, tanning beds, radiotherapy in childhood, breast cancer, thyroid cancer, and renal transplantation.

There are four clinical subtypes of melanoma. Superficial spreading melanoma arise (30%) from pre-existing melanocytic naevae. The horizontal growth phase through the epidermis is followed by vertical growth. A nodular melanoma means there is no horizontal growth. Lentigo maligna melanoma is seen in mainly elderly patients. Acro lentigenous starting on feet or hands is most common in Afro-Caribbeans.

Epidemiology
There is little data on skin cancer from the UK. There are 750,000 new basal cell carcinomas per year in the USA with incidence doubling every 10 years. There are 200,000 squamous cell carcinomas in the USA each year with 1300 to 2300 deaths resulting from non-melanoma skin cancer. There are 50,000 new cases of melanoma in the USA per year and 10,000 deaths.

In 1999 in the UK, there were 5,000 cases of melanoma. The incidence is increasing more rapidly than any other malignancy except lung cancer in women. It is the second most common cancer in young women. The incidence rose by 84% between 1980 to 1989. In the USA there is a lifetime risk of 1 in 32. There were 1378 deaths from melanoma in 1997.

Ultraviolet light exposure is the single most important factor in the pathogenesis of skin cancer. Skin cancer is commoner in Fitzpatrick skin type I individuals with sun sensitive skin. Skin cancer is commoner in populations living nearer the equator. Skin cancer is commoner in those working out of door.

Ultraviolet Light
The sun emits the entire electromagnetic spectrum but only the wavelengths between 290 and 3000nm reach the Earth’s surface. The carcinogenic potential of light is inversely related to its wavelength. The lowest wavelength to penetrate to the Earth’s surface is ultraviolet light which represents 10% of terrestrial sunlight.
Epidermis provides our first line of defence against harmful solar UV radiation. It is exposed to much higher levels of solar radiation than any other organ. This exposure increases the risk to skin cells of damage by UV and to DNA of UV-mediated mutagenesis and consequently to UV-induced cancer.

UVB is the most important wavelength in skin carcinogenesis, although 100 times more UVA penetrates to the Earth’s surface. UVA is the major cause of skin ageing, and will also contribute to skin carcinogenesis. UVA is used therapeutically in PUVA therapy, and can induce squamous cell carcinoma.

Photon wavelengths 250 to 300nm are absorbed by ring structures and linear repeats found in DNA and other complex macromolecules such as proteins. Energy absorbed by bonds in these structures makes them highly reactive and easily modified or broken. As UVC is mostly filtered out by the atmosphere, primarily UVB is responsible for this kind of DNA damage.

UVB induces well recognised abnormalities in DNA by a direct effect. Cellular DNA acts as a chromophore for UVB. It affects pyrimidine Cytosine (C) & Thymine (T) bases, most commonly 6-4 pyrimidones from pyrimidines, also formation of cyclobutane pyrimidine dimers from two adjacent pyrimidines e.g. -T-T-, -C-T-.

Photon energy can cause many molecules in the cell to become reactive. Releasing, for example, highly reactive free radicals that cause oxidative damage to DNA, proteins, etc. (contribute to photo-ageing of skin).

Where one of the two strands of is damaged, DNA can be removed and replaced by the cell’s normal nucleotide excision repair system. In Xeroderma pigmentosum this pathway is inactive because of mutations in genes encoding repair enzymes, so DNA damage is unrepaired and this leads to skin cancer if mutated cells persist.

“UV signature mutations” are where structurally-altered C and T bases are “misread” by DNA repair and replication enzymes and replaced with different bases. For example, damaged CC in DNA replaced by TT, or damaged CT replaced by TT. Thus, the coding sequence of a gene is altered, the mRNA is changed and this is translated into a protein with an altered function or which does not function at all or the mRNA may not even be transcribed fully so no protein is made.

Immune Function
Immune surveillance within the skin is provided by the Langerhans cell. They are migrants to the epidermis developing from CD34 bone marrow precursors. Langerhans cells are distributed in the suprabasilar layer of the epidermis accounting for 1 to 2% of the epidermal cells. Langerhans cells have complex dendritic processes which cover 25% of the skin surface.

Langerhans cells will recognise tumour associated antigens on developing skin cancer cells. They will process the antigen and present this to T cells inducing cell mediated cytotoxicity, destroying the cancer cells before a skin cancer can develop.

Langerhans cells are sensitive to UV damage, and following UV exposure, the density of Langerhans cells decreases. Langerhans cell function decreases in a dose dependent manner with UV irradiation. Developmentally this may be important in preventing the development of autoimmune disease to neoantigens induced in the skin by UV exposure.

An efficient immune system is important for defeating skin and other tumours early in their development. In individuals who are immunocompetent, many very early cancers are probably resolved (unknown to and undetected by us) very early by our own immune systems.
In patients who are **immunosuppressed** - either through long term immunosuppressive therapy (e.g. transplant patients or patients with severe psoriasis) or through disease (e.g. HIV infection); there is an **increased occurrence of keratinocyte-derived skin tumours** on sun-exposed sites.

UVA and UVB light can affect the **expression of genes** that are involved in various aspects of normal skin immunity, resulting in reduced skin immunocompetence. This is the basis of some forms of UV phototherapy for treatment of some inflammatory skin diseases like psoriasis.

**UV exposure increases expression of some down-regulatory cytokines such as interleukin 1Ra, which suppresses Langerhans cell activity.** It also **inhibits expression of adhesion molecules** such as ICAM-1 (inflammatory cell adhesion molecule 1) and **decreases the migration of T cells**, etc. into the skin. UV radiation **depletes the number of Langerhans cells** in the epidermis, decreasing epidermal immuno-surveillance.

**UV-induced immunosuppression** combined with the ability of UVB to cause cancer-generating mutations further increases the cancer-causing potential of sun exposure.

**The Role of p53**

**p53** is a 55kDa nuclear phosphoprotein encoded on the short arm of chromosome 17. It binds to DNA as a tetramer with activation of genes involved in cell differentiation, proliferation, induction of DNA repair and apoptosis. It is present at very low levels in normal cells. Following a biological challenge, levels increase due to post-translational protein stabilisation.

**Elevated levels** of p53 mediates growth arrest in the G1 phase by inducing the transcription of p21\textsuperscript{waf1, cip1} cell cycle regulatory cyclins. p21 **inhibits proliferating cell nuclear antigen** (PCNA), and this **inhibits activation of cyclin dependent kinases** and thus cell cycle regulatory cyclins.

If the damage is not repairable, the cell is induced to undergo **apoptosis** forming a sun-burn cell. This is mediated by activation of the human Bax gene transcription which heterodimerises anti-oxidant Bcl-2 associated with mitochondrial membranes, promoting apoptosis.

The p53 gene is sensitive to UV damage. Mutation of one allele of the p53 gene will lead to the production of mutant p53 which inactivates the wild type p53. Failure of p53 to arrest cell division leads to division of the cells with daughter cells acquiring the DNA mutation.

Mutations of p53 are common in **squamous cell carcinomas**. Mutations are seen in up to 85% of **melanomas**. Mutations are low in a primary melanoma but are high in a metastatic melanoma, so this is possibly not an early event in melanoma tumorigenesis.

Mutations that can start a cell down the route to becoming cancer include:

1. Mutations that stimulate **uncontrolled cell proliferation** e.g. by abolishing control of the normal “cell cycle” (p53).
2. Mutations which **alter responses to growth stimulating/repressing factors** by altering structures of signalling pathway proteins e.g. pathways permanently signalling “proliferate” or rendering cells “blind” to inhibitory signals.
3. Mutations that **inhibit programmed cell death** (apoptosis).

Mutations in genes involved in these types of pathways may initiate or continue the pathway towards a keratinocytes or melanocytes becoming malignantly transformed.

**Mutations leading to failure of normal programmed cell death** are generally considered to be one of the most important events in triggering transformation of a healthy cell into a cancer cell. Cells that are damaged beyond repair by normal mechanisms (e.g. by UVB) usually undergo **apoptosis**. It is an important pathway by which the immune system kills “unusual” (e.g. cancer) cells.
Apoptosis is a normal part of our biology. It is a process by which cells are renewed or replaced and by which organs are shaped during development.

In the normal epidermis, the end stage of keratinocyte differentiation is programmed cell death.

**The apoptotic cell undergoes a series of defined steps:**

1. Severe DNA or protein damage.
2. Withdrawal of survival/factors.
3. Binding of specific ligands to specific cell surface receptors (e.g. Fas/Fas ligand) also known as CD95/CD95 ligand.

These events can all trigger a cascade of intracellular signals that initiate the programme. Mutations which affect genes involved in or in responding to any of these trigger types can result in failure of a cell to follow the apoptosis programme.

**Apoptosis** is characterised by cytoskeletal disruption, cell shrinkage & formation of apoptotic cell envelope, cell membrane blebbing, and nuclear fragmentation – DNA broken down into oligonucleosome fragments. Apoptosed cell phagocytosed by surrounding cells or breaks up into apoptotic cell bodies. There is no inflammatory response.

**Necrosis** is characterised by changes that include membrane damage, cell swelling, release of cell contents; all of which are combined with an inflammatory response.

In sunburn, UV leads to **keratinocyte cell apoptosis**. “Sunburn Cells” are apoptotic cells in UV overexposed skin. Apoptosis removes UV damaged cells in the skin which otherwise might become cancer cells. **UVA** causes formation of free radicals: oxygen or superoxide anions that can damage mitochondrial membranes. This damage triggers apoptosis. **UVB** induced DNA damage stimulates apoptosis: e.g. DNA damage activates transcription factor p53 and AP1. **p53** stimulates expression of the Bax gene which initiates the apoptosis pathway. **AP1** stimulates expression of Fas ligand. **FasL** binds to Fas which initiates apoptosis.

**Mutations in p53** have been associated with keratinocyte cancers. Failure of p53 expression results in loss of one DNA damage-activated pathway, and so damaged or mutated & potentially cancerous cells may survive.

**Mutations which lead to Fas L over-expression** has been found in malignant melanoma and other cancers. Expression of FasL enables the malignant cells to avoid immune attack. The FasL binds to Fas-bearing lymphocytes triggering lymphocyte apoptosis.

**Host Response to UV**

The response to UV exposure by the individual is dictated by genetic influences. Skin phototype is genetically determined. A number of genetic disorders will influence the way the body reacts to UV light and the risk of skin cancer. **Fitzpatrick Phototypes** are I-VI, from skin that always burns and never tans, to Afro-Caribbean skin of a marked constitutive pigmentation.

**Melanin** pigmentation is responsible for skin colour. Melanin is produced by **epidermal melanocytes** within the basal layer of the epidermis. Different skin colours depend on the amount and type of melanin produced, not the density of melanocytes which is fairly constant.

Melanocytes are of neural crest origin. They are dendritic and melanin is distributed throughout the epidermis within keratinocytes. Each melanocyte is associated with 36 keratinocytes forming the epidermal melanin unit. Melanin is packaged into melanosomes and transferred to keratinocytes via the dendrite tips which are phagocytosed by the keratinocytes.

There are two types of melanin formed: **eumelanin** (brown or black and insoluble) or **phaeomelanin** (yellowish or reddish brown and soluble in alkalis, found in red-heads). Melanin is formed from tyrosine via a series of enzyme reactions.
Melanin is a chromophore for UV light, absorbing it and thus providing protection for the most sensitive lower levels of the skin. Melanin also traps electrons and free radicals. Melanin has an adaptive significance in that constitutive skin pigmentation is highest in the tropics and lowest in temperate climates with some increase in Northern races subjected to prolonged snow glare.

Cutaneous protection against UV exposure includes tanning (immediate due to alteration and redistribution of melanin or delayed with new melanin production), epidermal thickening, and sunburn (discourages further sun exposure).

**Genetics and Skin Cancer**

**Basal cell naevus syndrome**, for example, is autosomal dominant. Affected individuals develop basal cell carcinomas in the second decade, often on the trunk. It is associated with palmar pit and skeletal abnormalities like bifid ribs and dentigerous cysts. It requires mutation of both alleles of the **Patched gene**. Mutations of this gene have been identified in sporadic basal cell carcinoma.

**Xeroderma pigmentosum** is an autosomal or sex linked recessive disease where there is a defect in the nucleotide excision repair mechanism. Affected individuals show solar ageing of the skin in the first years of life with freckling. Dysplastic and neoplastic skin changes occur in the first decade with basal cell carcinomas, squamous cell carcinomas and melanomas (in Caucasians). Death results in those severely affected, and this occurs in the second decade from metastatic skin cancer.

**Epidermodysplasia verruciformis** is an autosomal recessive disease associated with mild immunodeficiency - reduced CMI. Affected individuals develop multiple viral warts in the first decade of life, forming extensive plaques. The human papilloma types are characteristically HPV5, 8, 9, 12, 14, 15, 17, 19-25, 28, 29, 36, 37, 38, 47, 49 and 50. These viruses are oncogenic and following sun exposure, infected keratinocytes undergo malignant transformation to squamous cell carcinomas. The E6 protein of HPV has been shown to interfere with DNA repair following UV exposure. E6 protein inactivates p53 allowing damaged cells to proliferate.

There are links between sporadic squamous cell carcinoma and HPV. In *epithelioma caniculatum*, viral particles have been identified on electronmicroscopy. There is also a link in renal transplant recipients (drug induced immunosuppression to preserve the transplant) as squamous cell carcinoma are 10 times more common than basal cell carcinoma ad EV-HPV types have been identified from squamous cell carcinomas.

**Dysplastic naevus syndrome** (Familial Atypical Mole/Melanoma FAMM syndrome) is where multiple dysplastic naevae are associated with multiple primary melanomas. In 40-50% of cases germline mutations of CDKN2A are detected. CDKN2A on chromosome 9 codes for p16, a cyclin kinase regulating cell division.

**CDKN2A** gene encodes for **p16**, a cyclin dependent kinase inhibitor. p16 promotes cell cycle arrest. Mutations inactivate the genes allowing proliferation of the cell. CDKN2A is also implicated in gallbladder adenocarcinoma.

Homoyzgous deletions or mutations of CDKN2A genes have been detected in up to 75% of melanoma lines. All in situ melanomas and most primary melanomas retain p16. 72% of metastatic melanomas show partial or complete loss of p16, so it could be more related to invasiveness than to tumour promotion.

**Prevention of Skin Cancer**

There are campaigns to increase public awareness of the dangers of sun exposure. It is important to use sun-blocks to protect the skin and to use sun protective clothing and hats. Increasing public awareness is important to try to identify skin cancer at an early stage when surgical removal may be curative.

**Sun blocks** are agents which either reflect UV or absorb and quench UV energy. Reflectant sun blocks include titanium dioxide or zinc oxide, which are cosmetically not the most acceptable. Absorvent sun blocks absorb UV energy and dissipate it as heat - various chemicals are used which have activity in UVA and UBV ranges.
Sun Protection Factor (SPF) is the time taken in the sun with a sun block before skin redness develops 24 hours later divided by the time taken in the sun without sun block before skin redness develops 24 hours later. In the UK, SPF 30 should be used through the summer, but if going to sunnier climes this should be increased to SPF 60.

An extensive range of sun protective clothing is now available, particularly for children which should be a target population. Hats should be solid cloth (not Straw hats) and with a broad brim to protect the forehead and nose. Stockings only have an SFF of 2 and we are now seeing a large number of older women with severe solar damage to the backs of the legs.

Learning Objectives for this lecture:
- Summarise the epidemiology of skin cancer.
- Explain the pivotal role of ultraviolet light in the pathogenesis of skin cancer.
- Describe the effect of ultraviolet light on DNA and on immune function in the skin.
- Explain the role of the tumour suppressor gene p53 in relation to UV-induced mutations.
- Describe the role of oncogenic human papilloma virus in the pathogenesis of squamous cell carcinoma (genetic model of epidermodyplasia verruciformis and renal transplant recipients).
Breast cancer is the leading female cancer, accounting for almost 1 in 5 cancer deaths among women. Each year in the UK over 44,000 women are diagnosed with breast cancer - that’s over 100 per day. Breast cancer shows a 1 in 9 life time risk. In the last 10 years, breast cancer rates in the UK have increased by 12%. 8 in 10 breast cancers are diagnosed in women aged 50 and over. It was the commonest cancer in the UK and Europe (even though it affects only 1 gender) in 2006 and it out-ranked lung cancer for the 1st time. In the 1970s around 5 out of 10 breast cancer patients survived beyond five years, but quite significantly now it is 8 out of 10.

In humans, the mammary glands undergo significant changes post-natally. The key hormonal cues occur in puberty, and the two steroid hormones are oestrogen and progesterone. Oestrogen plays a lead role in growth of mammary gland tissue. Oestrogen receptor positive breast cancers respond to oestrogen. The mammary glands are a ductal network of epithelial cells forming a grapevine structure, embedded in stromal fatty tissue. This network can undergo further changes following hormonal cues to produce milk. The primary job is to duct milk to the nipple.

Cancers of all cell types occur in breasts. For example, a very rare type originates from the stroma called a phyllodes tumour. These are large, fast growing, extremely aggressive tumours. More common are cancers that originate from the luminal epithelial cells of the ductal network.

The organised architecture is lost in cancer. In mammary glands there are two layers of epithelial cells lining the lumen - myoepithelial cells and luminal epithelial cells.

Myoepithelial cells are thought to squeeze the lumen of the cells, but competing theories show that they are important in forming the tubule structure. There is a subclass of breast cancers that arise from myoepithelial cells, known now as oestrogen receptor negative type breast cancers.

The luminal epithelial cells line the lumen, and these are the precursor cell types for most breast cancers. This population of cells are usually 15-30% positive for oestrogen receptors.

Some patients present with benign / in situ carcinoma, where there is a proliferation of cells within the tubular network. This is a pre-cancerous state that we can pick up. This state gives rise to the further five forms of breast cancer we know of. Of those, about 80% can become breast carcinomas. Histologically, there is very little to show that these came from an organised tubular structure. Lobular carcinomas form about 15% of breast cancers, and can still bear some resemblance to the tubular structure from which they arise. Breast cancers with a very unusual pathology are medullary carcinomas, packed full of vessels.

Major histological types of invasive breast cancer include infiltrating ductal carcinoma (IDC), many of which feature no special type of histological structure and account for almost 80% of breast cancers. Tumours in this class can metastasise to axillary lymph nodes, and their prognosis is the poorest of the various tumour types.

In 1889, Albert Schinzinger noted that atrophy of the breast follows cessation of ovarian function and proposed ovariectomy as a treatment for breast cancer. In 1896 George Beatson demonstrated that ovariectomy in pre-menopausal women resulted in disease regression and improved prognosis. Subsequent studies confirmed that ovarian hormones stimulate breast cancer development and identified oestrogen as the hormone responsible.

Important risk factors include lifetime of exposure to oestrogens: age of onset of menarche, age to first full-term pregnancy, some contraceptive pills, and some hormone-replacement therapies.
Some breast cancers like normal breast are sensitive to the effects of oestrogen. Approximately one third of pre-menopausal women with advanced breast cancer will respond to **oophorectomy**. Paradoxically, breast cancer in post-menopausal women responds to high-dose therapy with synthetic oestrogens (causes breast tumour regression).

**Oestrogen receptor (ER)** is expressed in around 50% of breast cancers. Presence is indicative of a better prognosis. The oestrogen receptor is **intracellular**. When it binds oestrogen, it translocates itself to the nucleus and acts as a transcription factor. In ER-positive cases, oestrogen regulates the expression of genes involved in cellular proliferation leading to breast cancer. Oestrogen withdrawal or competition for binding to ER using anti-oestrogens results in a response in about 70% of ER-positive cancers, 5-10% of ER-negative cancers also respond. An increased level of expression of ER indicates a good prognosis in female breast cancer but a worse prognosis in male breast cancer.

The oestrogen receptor is activated upon binding oestrogen. Gene expression is induced by binding to specific DNA sequences called **Oestrogen Response Elements**. The oestrogen-induced gene products increase cell proliferation, resulting in breast cancer.

**Treatment**
The main treatment for breast cancer is **surgery**. Removal of the tumour is a curative step. In addition radiation therapy and chemotherapy are used, and in breast cancers that are oestrogen responsive, we can also use **endocrine therapy**.

Endocrine therapy is the cornerstone of breast cancer treatment. This can be achieved at the levels of **ovarian suppression** (removing the ovaries or destroying them by radiation), **blocking the production of oestrogen** by enzymatic inhibition, and **inhibiting oestrogen responses**.

**Ovarian Suppression**
Firstly, what are the sources of oestrogen in the body? LHRH (GnRH) is produced by the hypothalamus, the pituitary gland secretes LH and FSH, and in pre-menopausal women, these stimulate the ovaries to make oestrogen. The pituitary also makes ACTH to stimulate the production of adrenal androgens, which can be peripherally converted to oestrogen.

In **ovarian ablation**, the aim is to limit or remove the effects of ovarian oestrogen. The ovary is the major source of oestrogen biosynthesis in pre-menopausal women. Ovarian ablation can be carried out by **surgical oophorectomy** or **ovarian irradiation**. The major problems associated with these procedures are morbidity and irreversibility. To overcome these issues, treatments to produce medical ovarian ablation have been developed.

Reversible and reliable medical ovarian ablation can be achieved using **LHRH agonists**. LHRH agonists bind to LHRH receptors in the pituitary leading to the **down-regulation and suppression of LH release** and inhibition of ovarian function, including oestrogen production. LHRH agonists include Gosrelin, Buserelin, Leuprolide and Triptorelin. In addition to LHRH agonists, medical treatments also include **aromatase inhibitors** and **anti-oestrogens**.

**Anti-oestrogens** are directly inhibitory of oestrogen action. Tamoxifen is a compound that is able to act like oestrogen and bind to oestrogen receptors. ICI 182 780 is another compound (more like oestrogen in structure) that has the same antagonistic effect. As early as 1958, non-steroidal anti-oestrogens were described and their clinical use was explored. By the 1960s the pharmaceutical industry had synthesised a wide range of anti-oestrogenic compounds. Only Tamoxifen (ICI 46474, Nolvadex) was developed further because of demonstrated efficacy and a low incidence of side effects.
**Tamoxifen** is a competitive inhibitor of oestradiol binding to the ER. Anti-oestrogens negate the stimulatory effects of oestrogen by blocking the ER, causing the cell to be held at the G1 phase of the cell cycle. **Tamoxifen is the endocrine treatment of choice for metastatic disease in post-menopausal patients** (approximately a third of patients respond). It has few side effects, only hot flushes were reported (29%) during therapy.

Tamoxifen is of a class of drug known as **selective estrogen receptor modulators (SERMs)**.

In **osteoporosis**, oestrogen is important to maintain bone in pre-menopausal women. After menopause, hormone replacement therapy is often recommended to prevent the development of osteoporosis. Clearly, the long-term administration of an anti-oestrogen has the potential to precipitate premature osteoporosis. However, **Tamoxifen actually has oestrogenic effects in bone**.

In **atherosclerosis**, oestrogen lowers LDL cholesterol levels and raises HDL levels. Following menopause, women are at the same risk for coronary heart disease as men. It can be argued that the long-term administration could produce a population at risk for premature coronary heart disease. **Tamoxifen actually has oestrogenic effects in the cardiovascular system**.

However, undesirably, anecdotal reports associate the administration of Tamoxifen for advanced breast cancer with subsequent thromboembolic episodes. Tamoxifen is known to produce endometrial thickening, hyperplasia, and fibroids following several years of therapy.

Tamoxifen is agonistic in bone and in the cardiovascular system, while antagonistic in breast cancers. The reason for this is complex and thought to be due to the mechanism by which the oestrogen receptor works in transcription. In breast cancer, Tamoxifen binding causes a conformational (shape) change in the oestrogen receptor that allows it to interact with proteins called "transcriptional co-repressors", thereby inhibiting oestrogen regulated gene expression. In tissues where Tamoxifen acts as an agonist, the same conformational change favours interaction with "transcriptional co-activators", as opposed to transcriptional co-repressors, thereby stimulating estrogen regulated gene expression.

The success of Tamoxifen in the treatment of all stages of breast cancer has focused attention on the possibility of developing additional drugs with different pharmacologic properties. Three compounds, Toremifene, ICI 182 780, and Raloxifene merit comment at present.

**Toremifene (Farnesdon)**, is a structural derivative of Tamoxifen with similar anti-oestrogenic and estrogenic properties.

**ICI 182 780** (Faslodex or Fulvestrant) exhibits no oestrogen-like effects in laboratory tests, but it is effective in controlling oestrogen-stimulated growth. Faslodex is a “pure anti-oestrogen” and may offer clinical advantages over Tamoxifen by decreasing tumour cell invasion and the stimulation of occult endometrial carcinoma. Overall, this class of drugs could have a role as a first-line therapy for advanced breast cancer and as a second-line therapy in patients in whom primary Tamoxifen treatment fails. It was licensed in the UK for advanced breast cancer in 2004.

**Raloxifene (Evista)** is an anti-tumour agent in animals. Raloxifene is agonistic in bone, with no activity in breast and uterus. Raloxifene is used in the treatment of osteoporosis in post menopausal women.

Tamoxifen reduces the incidence of contralateral breast cancer by 50%. This has led to clinical trials for breast cancer prevention. Trials have focussed on “high risk” patients, e.g. those who have had previous benign breast pathology (5 years) or those with previous family history. Tamoxifen vs placebo trials have shown a 38% reduction in overall breast cancer incidence, no effect on ER negative breast cancer incidence and no association between prevention and patient age.

Problems associated with using Tamoxifen in prevention include increased incidence of endometrial cancer, stroke, deep vein thrombosis, and cataracts. To overcome these problems, prevention trials are being conducted with Faslodex (SERM) and aromatase inhibitors.
Blocking Oestrogen Production by Enzymatic Inhibition

In post-menopausal women, the major source of oestrogen derives not from the ovaries but from the conversion of the adrenal hormones androstenedione and, to a lesser extent, testosterone to oestrone. This enzymatic conversion occurs at extra-adrenal or peripheral sites such as fat, liver and muscle. This conversion is catalysed by the aromatase enzyme complex.

Aromatase consists of a complex containing cytochrome P450 heme containing protein as well as the flavoprotein NADPH cytochrome P450 reductase. Aromatase catalyses three separate steroid hydroxylations involved in the conversion of androstenedione to oestrone. Aromatase can metabolise androstenedione, which is produced by the adrenal glands. This leads to the production of oestrone sulphate, which is circulated in the plasma.

A convenient classification divides the aromatase inhibitors into the mechanism-based, or suicide, inhibitors (type I) and those that are competitive inhibitors (type II).

Suicide inhibitors initially compete with the natural substrate (i.e. androstenedione and testosterone) for binding to the active site of the enzyme. The enzyme then specifically acts on the inhibitor to yield reactive alkylating species, which form covalent bonds at or near the active site of the enzyme. Through this mechanism, the enzyme is irreversibly inactivated.

An example of this type of drug is “Exemestane”. Single-dose administration reveals a major reduction of plasma oestrogens with this compound. Side effects associated with Exemestane treatment were mild and included hot flushes, nausea, and fatigue.

Competitive inhibitors bind reversibly to the active site of the enzyme and prevent product formation only as long as the inhibitor occupies the catalytic site.

An example of this type of drug is “Anastrozole” (“Arimidex”: ICI-D1033). Studies demonstrate suppression of plasma oestrogen to levels approaching the limits of assay sensitivity. Anastrozole was the first aromatase inhibitor to be approved in the United States for the management of advanced breast carcinoma in postmenopausal women.

Progesterone is the dominant naturally occurring progestin. The poor absorption of progesterone has been overcome with some of the synthetic derivative progestins. The progestin response in human breasts is complex and influences both proliferation and differentiated function. Progestins are used in endocrine treatment of uterine and breast cancer with clinically proven anti-neoplastic properties. Progestin therapy for
metastatic breast cancer has been used principally as a second or third line therapy following selective oestrogen. The principal progestin used for metastatic breast cancer has been Megestrol Acetate.

A significant proportion of patients presenting with breast cancer and all patients with metastatic disease become resistant to endocrine therapies. However, most cases continue to demonstrate oestrogen responses and contain oestrogen receptor.

In treatment, 60% of ERα-positive tumours respond to endocrine therapy. These include anti-oestrogens like Tamoxifen, and inhibitors of oestrogen synthesis like Exomestrane. The clinical problem is the initial response but eventual relapse due to resistance to prolonged endocrin therapy, not due to tumours becoming ER independent. The solution is to continue to use endocrine therapies as these are successful, but there is the requirement for additional therapeutic agents.

The breast cancer screening programme uses mammography to screen all women between 50 and 64 who are registered with a GP in the UK. The screening age is being extended to age 70 across the country. Each patient is asked to attend for a test once every 3 years. More than 70% of women attend breast screening appointments. This means that more than 1,200,000 women are screened for breast cancer each year in the UK. Only 6 out of every 100 are asked to go back for more tests. More than 90% of breast tumours are first spotted by women themselves.

Established risks of breast cancer include age, family history, early age of menarche, late menopause, having no children, a history of benign disease, and lobular carcinoma in situ.

Possible risks include the contraceptive pill, HRT, diet, weight, alcohol, and being tall.

Learning Objectives for this lecture:
- Summarise information on the incidence of breast cancer.
- Describe the histology and clinical features of breast cancer.
- Describe breast cancer as a steroid hormone regulated disease.
- Explain the nature of endocrine therapies in breast cancer.
- Summarise what is known about the genetics of familial breast cancer.
- Briefly describe the consequences of metastasis of breast tumours.
**Leukaemia**
by Professor Barbara Bain

Leukaemia is *cancer of the blood* - this is 5% of all cancers. In the UK approximately 60 people every day are diagnosed with cancer of the blood. Blood cancers are the most common cancers in men and women aged between 15 and 24, and are the main cause of cancer death in people aged between 1 and 34. Unfortunately, 1 in 45 of the UK population will die of leukaemia, lymphoma or myeloma.

Leukaemia means “**white blood**”, and this name was given because the first cases of leukaemia recognised had a marked increase in the white cell count. Leukaemia is actually a **bone marrow disease** and not all patients have abnormal cells in the blood.

Leukaemia results from a **series of mutations** in a single lymphoid or myeloid **stem cell**. These mutations lead the progeny of that cell to show abnormalities in proliferation, differentiation or cell survival leading to steady expansion of the leukemic clone. Any of the cells on the right can be involved.

**Why Leukaemia is Different**
Leukaemia is different from other cancers. Most cancers exist as a **solid tumour**, but in leukaemia it is uncommon for patients to have tumours. More often they have leukaemic cells replacing normal bone marrow cells and **circulating freely** in the blood stream. Leukaemia is different from other cancers because haemopoietic and lymphoid cells behave differently from other body cells. Normal haemopoietic stem cells can **circulate** in the blood and both the stem cells and the cells derived from them can **enter tissues**. Normal lymphoid stem cells recirculate between tissues and blood.

The concepts of invasion and metastasis cannot be applied to cells that normally travel around the body and enter tissues. We have to have other ways of distinguishing a **benign** condition from a **malignant** condition, and haematologists usually use different words for these concepts.

Leukaemias that behave in a relatively **benign** manner are called **chronic**, which means the disease goes on for a long time. Leukaemias that behave in a **malignant** manner are called acute, which means that unless treated the disease is very aggressive and the patient dies quite rapidly.

Following on from **acute** or **chronic** leukaemia, the disease can be classified as **lymphoid** or **myeloid**. Lymphoid can be of B or T cell lineage. Myeloid can be any combination of granulocytic, monocytic, erythroid or megakaryocytic.

**Put all this together and we get:**
- **Acute lymphoblastic leukaemia** (ALL)
- **Acute myeloid leukaemia** (AML)
- **Chronic lymphocytic leukaemia** (CLL)
- **Chronic myeloid leukaemia** (CML)

**Aetiology of Leukaemia**
Leukaemia results from a **series of mutations in a single stem cell**. Some mutations result from identifiable (or unidentifiable) oncogenic influences. Others are probably random errors (chance events) that occur throughout life and accumulate in individual cells. The important leukaemogenic mutations that have been recognised include **mutation in a known proto-oncogene**, creation of a **novel gene** (e.g. a chimaeric or fusion gene), or **dysregulation** of a gene when translocation brings it under the influence of the promoter or enhancer of another gene. Point mutations are common.

Loss of function of a **tumour suppressor gene** can also contribute to leukaemogenesis - this can result from deletion or mutation of the gene. If there is a tendency to increased chromosomal breaks, the likelihood of
leukaemia is increased. In addition, if the cell cannot repair DNA normally, an error may persist whereas in a normal person the defect would be repaired.

Inherited or other constitutional abnormalities can contribute to leukaemogenesis, for example Down's syndrome, chromosomal fragility syndromes, defects in DNA repair, and inherited defects of tumour suppressor genes.

Identifiable causes of leukaemogenic mutations include irradiation, anti-cancer drugs, cigarette smoking, and chemicals like benzene. In Hiroshima, the likelihood of leukaemia occurring is related to the distance of the person living to where the bombs were dropped - this is a dose relationship. There is strong evidence that benzene causes leukaemia, as this is seen in industrial workers.

Leukaemia, like cancer in general, can be seen as an acquired genetic disease, resulting from somatic mutation. Mutation in germ cells may bring favourable, neutral or unfavourable characteristics to the species. Somatic mutation may be beneficial, neutral or harmful. Beneficial mutations are rare but can lead to reversion to the normal phenotype in some cells in individuals with an inherited abnormality, e.g. an immune deficiency or bone marrow failure syndrome.

Since some mutations that contribute to leukaemogenesis appear to be random events rather than caused by an exogenous influence, they may result from the nature of the human genome. Leukaemia may thus be, in part, the inevitable result of the ability of mankind to change and evolve.

The Difference between Acute and Chronic Myeloid Leukaemia

In acute myeloid leukaemia, cells continue to proliferate but they no longer mature so there is a build up of the most immature cells (myeloblasts) in the bone marrow with spread into the blood. There is therefore also a failure of production of normal functioning end cells like neutrophils, monocytes, erythrocytes and platelets.

In acute myeloid leukaemia, the responsible mutations usually affect transcription factors so that the transcription of multiple genes is affected. Often the product of an oncogene prevents the normal function of the protein encoded by its normal homologue. Cell behaviour is profoundly disturbed.

In chronic myeloid leukaemia, the responsible mutations usually affect a gene encoding a protein in the signalling pathway between a cell surface receptor and the nucleus. The protein encoded may be either a membrane receptor or a cytoplasmic protein.

In chronic myeloid leukaemia, cell kinetics and function are not as seriously affected as in acute myeloid leukaemia. However, the cell becomes independent of external signals, there are alterations in the interaction with stroma and there is reduced apoptosis so that cells survive longer and leukaemic clone expands progressively. Whereas in acute myeloid leukaemia there is a failure of production of end cells, in chronic myeloid leukaemia there is increased production of end cells.

Acute lymphoblastic leukaemia has an increase in very immature cells (lymphoblasts) with a failure of these to develop into mature T and B cells. In chronic lymphoid leukaemias, the leukaemic cells are mature, although abnormal, T cells and B cells.

Disease Characteristics of Leukaemia

Accumulation of abnormal cells leads to leucocytosis, bone pain (if leukaemia is acute), hepatomegaly, splenomegaly, lymphadenopathy (if lymphoid), thymic enlargement (if T lymphoid), skin infiltration.

Metabolic effects of leukaemic cell proliferation include hyperuricaemia and renal failure, weight loss, low grade fever and sweating.

Crowding out of normal cells lead to anaemia, neutropenia and thrombocytopenia. The hand of a patient with acute myeloid leukaemia may show bruising and oedema. A CT scan of a patient with acute myeloid leukaemia may show intraventricular haemorrhage. Leukaemic cells migrate into places where bacteria are found by chemotaxis, for example in the gums. Loss of normal immune function as a result of loss of normal T cell and B cell function is a feature of chronic lymphoid leukaemia, so opportunistic infections are common.
**Epidemiology**

Acute lymphoblastic leukaemia is largely a disease of children. Epidemiology suggests that B lineage all may result from delayed exposure to a common pathogen. Evidence relates to family size, new towns, socio-economic class, early social interactions and variations between countries. Epidemiology also suggests that some leukaemias in infants and young children result from irradiation in utero or in utero exposure to certain chemicals like Baygon or Dipyrene. Leukaemia rarely all results from exposure to a mutagenic drug.

Clinical features of acute lymphoblastic leukaemia results from accumulation of abnormal cells, and patients present with bone pain, hepatomegaly, splenomegaly, lymphadenopathy, thymic enlargement and testicular enlargement.

Other features result from crowding out of normal cells. This leads to fatigue, lethargy, pallor, and breathlessness (caused by anaemia). It leads to fever and other features of infection (caused by neutropenia). It also leads to bruising, petechiae and bleeding (caused by thrombocytopenia).

**Haematological features** include leucocytosis with lymphoblasts in the blood, anaemia (normocytic, normochromic), neutropenia, thrombocytopenia, and replacement of normal bone marrow cells by lymphoblasts.

**Diagnosis**

**Investigations** include blood counts and films, check of liver and renal function and uric acid, bone marrow aspirate, cytogenetic/molecular analysis and a chest x-ray.

**Cytogenic and molecular genetic analysis** is useful for managing the individual patient because it gives us information about prognosis. Cytogenic and molecular genetic analysis advances knowledge of leukaemia because it has permitted the discovery of leukaemogenic mechanisms. Hyperdiploidy usually means a good prognosis.

Leukaemogenic mechanisms include formation of a fusion gene, dysregulation of a proto-oncogene by juxtaposition of it to the promoter of another gene (e.g. a T-cell receptor gene), and point mutation in a proto-oncogene.

Cytogenetics can be detected by two fluorescent probes, a green probe for ETV6 and a red probe for RUNX1; when a fusion gene is formed the two colours fuse to give a yellow fluorescent signal. This technique is called fluorescence in situ hybridisation - FISH.

**Treatment**

Acute lymphoblastic leukaemia treatment is supportive with red cells, platelets and antibiotics, and systemic chemotherapy and intrathecal chemotherapy are also used.

**Summary**

Leukaemia results from acquired somatic mutation in haemopoietic or lymphoid stem cells. A single cell gives rise to a clone of leukaemic cells that replace normal cells. Disease characteristics are due to proliferation of leukaemic cells and loss of function of normal cells.

Unlike other cancers, leukaemia is disseminated from very early in the disease process. Treatment must therefore be systemic.

Acute lymphoblastic leukaemia results from mutation in T or B lineage lymphoid stem cells. Acute lymphoid leukaemia is not a single disease or even two diseases. There are multiple different leukaemogenic mechanisms giving different disease phenotypes.

It is the molecular genetic events that determine the disease phenotype including the prognosis. This in turn determines the optimal treatment for individual patients. In the future, identification of the precise genetic events underlying the leukaemia is likely to indicate the best treatment for an individual patient.
Learning Objectives for this lecture:
- Explain what “leukaemia” is.
- Explain the difference between lymphoid and myeloid leukaemia.
- Explain the difference between acute and chronic leukaemias.
- Outline the clinical and haematological features and representative cytogenetic and molecular genetic abnormalities of acute lymphoblastic leukaemia.