Biomedical Science - Cancer

Hallmarks of cancer -

1. Resisting Cell Death
2. Sustaining proliferation signalling
3. Evading Growth Suppressors
4. Enabling Replicative Immortality
5. Deregulating Cellular Energetics
6. Genome Instability
7. Inducing Angiogenesis
8. Activating Invasion & Metastasis
9. Tumour Promoting Inflammation
10. Avoiding Immune Destruction

1. Resisting Cell Death:

Apoptosis – programmed cell death. Cancerous or infection development can provoke apoptosis in many ways, including genomic instability (DNA damage/mutations), hypoxia (lack of oxygen) before angiogenesis, lack of growth factors during metastasis and immune attack. Insufficient apoptosis can contribute to cancer development and resistance to anti-cancer treatment (as most treatments target apoptosis mechanisms).

Caspases - a family of protease enzymes playing essential roles in programmed cell death (including apoptosis, pyroptosis and necroptosis) and inflammation. These processes require both upstream and downstream caspases.

Apoptotic signalling -

- **Intrinsic, eg. Growth factor/ O2 deprivation or DNA damage -**
  - Bax (which is generally inactive in the cell) becomes activated, it moves to the mitochondria making the outer membrane permeable so that Cytochrome-C is released into the cytoplasm. Cytochrome-C along with Procaspase-9 and Apaf-1 form a apoptosome which then activates Caspase-9. Caspase-9 cleaves and activates pro-caspase-3 into caspase-3 which dismantles the cell causing apoptosis. Bcl-2 regulates cytochrome-c release by inhibiting Bax.
  - Upstream caspase – caspase-9
  - Downstream caspase – caspase-3
- **Extrinsic, eg. T-cell killing (TRAIL) -**
  - A 'death ligand' like TRAIL binds to the membrane receptor causing it to change conformation (form a cluster). Procaspase-8 is recruited to the intracellular domain of the receptor activating caspase-8, which can then go on to activate pro-caspase-3 into caspase-3 which dismantles the cell causing apoptosis. Caspase-8 can also indirectly activate Bax to amplify the mitochondrial pathway.
  - Upstream caspase – caspase-8
  - Downstream caspase – caspase-3

Eg. Follicular lymphoma - a type of non-Hodgkin lymphoma that develops when the body makes abnormal B-lymphocytes. It is generally a slow progressing disease but becomes very aggressive (“transforms”) in 45% of cases approx 6-7 years after diagnosis. In these cases the Bcl-2 gene is translocated from chromosome 18 to chromosome 14 and becomes associated with the IgH gene resulting in abnormally high levels of Bcl-2 expression in B cells (survival advantage). Ultimately there is an accumulation of cells with high levels of Bcl-2 (slow progression of FL) but following a secondary mutation, usually effecting proliferation, the cells are now able to divide quickly (now have a survival advantage and fast proliferation). The secondary mutation stimulates pro-apoptotic signals that would usually kill the cell but cannot due to high Bcl-2 levels. The cancer cells become 'addicted' to high Bcl-2 levels (as they would otherwise be killed) and when it is taken away they die (do not just lose their survival advantage).

- **Treatments:**
  - **ABT-737** – Targets Bcl-xL, closely related to Bcl-2 and therefor can also kill Bcl-2. Good affect at reducing cancer cells but has side effects (reduces platelet levels – Bcl-xL important in platelet survival). The pharmacological properties are also undesirable (cannot be orally administered).
  - **Navitoclax (ABT-263)** – Same specificity as ABT-737 targets (Bcl-xL, Bcl-2, Bcl-w) but can be administered orally (increased absorption). Same side effects were found.
  - **Venetoclax** – Only targets Bcl-2, not Bcl-xL therefor less toxic to platelets (will not target mutation in the Bcl-xL protein). Still oral bioavailable

Eg. Li Fraumeni Syndrome (LFS) - a rare cancer predisposition hereditary (autosomal dominant) disorder. Heterozygous (1 wildtype and 1 mutant gene) mutations in p53 are enough to cause at least one type of cancer in almost 100% of females and 75% of males (autosomal dominant). Spontaneous mutations of p53 can also cause tumours.

- p53 is the most commonly mutated gene in human cancers (>50%). p53 is a transcription factor which, as a tetramer, induces the expression of apoptotic proteins (eg. Bax activator – Puma) and promotes apoptosis. In healthy cells un-phosphorylated p53 is ubiquinated and readily degraded, but under stress/DNA damage active stress kinases can phosphorylate p53 up-regulating apoptosis (Puma). No current effective treatments targeting this mutation.
    - P53 acts as a tetramer. Most p53 mutations occur in the DNA binding region therefor once mutant tetramers are assembled they are unable to bind DNA and induce target genes. The mutation works in a dominant fashion therefor one mutant allele (1 mutant monomer) is enough prevent the protein from working properly (in heterozygous cells only 6% assemble with all 4 wildtype monomers)
- **Human papillomavirus –** There are over 100 different viruses, most are benign warts but others are able to trigger cancer development, especially in the cervix. HPV-16 and 18 cause ~70% of cervical cancers. Protein E6 of oncogenic HPV-16 and 18 strains contribute to their cancer promoting activity. HPV E6 promotes degradation of p53. Cervarix and Gardasil effectively protect against cervical cancer by preventing infection with HPV-16 and 18.

2. Sustaining proliferation signalling:

Growth factor signalling (ligand dependent proliferation) – In healthy cells this is a ligand dependent system. In the absence of the ligand, the receptor monomers of receptor tyrosine kinase (eg. Epidermal growth factor receptor EGFR) within the cell membrane are inactive. Binding of the ligand stabilises the dimerised form of the receptor and monomers become phosphorylated. An adaptor is recruited to the receptor which then recruits GDP-bound Ras which exchanges GDP for GTP. GTP-Ras binds Raf (eg. Braf) and promotes its phosphorylation causing a cascade of phosphorylation and activation of downstream kinases which can translocate into the nucleus. Ultimately this cascade activates transcription factors that induce expression of cell cycle proteins and cell proliferation. Ligand independent proliferation can occur when there are oncogenic alterations in the growth factor signalling pathway

- **Receptor Mutations:**
    - **eg. Glioblastoma -** Activation of receptor in the absence of the ligand or amplification of EGFR receptor or truncation of EGFR receptor (missing intracellular signalling portion). Ch806 (MAb806) is a chimetric antibody that has been designed to bind to the truncated or over expressed EGFR receptors, internalising the complex and resulting in degradation. Treatment was found to be safe and had good uptake (even into the brain). Tumour stopped growing but didn't kill the tumour, they are now working on producing an ADC.
    - **eg. Breast Cancer** – HER2 receptor is overactive in 20-25% of breast cancers, due to mutation, gene amplification and over expression. Herceptin is a humanised monoclonal antibody that can bind to HER2 and prevent its proliferation/survival signalling and shows positive results for treating breast cancer. HER2 is also overactive in approx 22% of gastric cancers but did not show nearly as much efficacy.
- **Ras Mutations:** A single base change which results in an amino acid change, at residue 12, can result in Ras being constitutively bound to GTP and therefor constantly active (even when ligand is not present). Most frequently mutated oncogene family in human cancers.
- **Raf Mutation:** presence of a mutation, usually V600E, in BRaf results in it being constitutively active (even when ligand is not present and Ras is inactive). Most melanomas bear the V600E mutation in BRaf which increases its kinase activity 10-fold.
    - **Zelboraf** – Compound that binds to the ATP binding site of the kinase. Drug was well tolerated and showed positive results in reducing the size of the tumours, but patients relapsed. Only prolongs life in those with the V600E mutation. 'Basket trials' were performed (a group of patients with V600E mutations were trialled regardless of cancer type – molecular profiling) and found mixed results.

3. Evading Growth Suppressors

Anti-growth signalling – In healthy cells this is a ligand dependent system. In the absence of the ligand, the TGF- receptor monomers within the cell membrane are inactive. Binding of the ligand stabilises the dimerised form of the receptor and they become phosphorylated. A Smad adapter protein is then recruited to the intracellular portions of the receptor and is phosphorylated. It can then interact with other Smad proteins forming a dimer which can then enter the nucleus and turn on the expression of cell cycle inhibitors. The most common problems associated with this pathway are either deletions or inactivations of the receptors or

Smad proteins (Smad deletion or mutations are associated with more invasive aggressive tumours). Currently no therapy for TGF- mutations.

- Some ovarian cancers have been found to contain TGF- mutations that result in the lack of expression of the protein.
- Smad deletions on their own do not cause cancer (do not require inhibition if there is not excessive proliferation)

Pancreatic cancer – generally a fairly rare cancer but has the lowest 5-year survival rate of all cancers.

- Approximately 90% of pancreatic cancers have activating Ras mutations (continuous proliferation), 75% have p53 mutations (prevents apoptosis) and around 55% (more aggressive tumours) have mutations/deletions in Smad proteins (prevent growth suppression).
- Smad deletions on their own do not cause cancer (do not require inhibition if there is not excessive proliferation) but when in individuals with a Ras mutation as well, this results in faster development of cancer than just Ras by itself.

4. Enabling Replicative Immortality

Telomeres – A structure at the end of the chromosome. During replication the telomeres are eroded and the chromosomes get shorter. Eventually this results in eroding of important DNA regions resulting in cell death.

Hayflick limit – the number of times a cell can divide before it undergoes senescence or apoptosis.

Telomerase – An enzyme that is able to restore telomere length by adding DNA after every cell cycle. Telomerase is expressed in embryonic stem cells and germ cells but not normal somatic cells. Cancer cells are able to reactive telomerase, bypassing the Hayflick limit and gaining indefinite replicative potential. Telomerase is composed of TERT (reverse transcriptase protein enzyme) and TERC (a RNA that provides a template for RT).

- There are three mutations in the promotor region of TERT that have been found to be highly associated with the development of cancer.
- Individuals with a TERT promotor mutation as well as a Ras or Raf mutation (combination of hallmarks) have a worse prognosis.

Telomerase inhibitors – Imetelstat is a lipid-conjugated oligonucleotide that is complimentary to, and binds with high affinity to, the RNA template (TERC) of telomerase. By binding to the TERC, it will be unable to be used as a template for RT and therefor telomere elongation will not occur. Imetelstat seems to be more effective against haemopoeitic (leukaemia etc) than solid tumours (lung cancer).

5. Deregulating Cellular Energetics – Normal cells in the presence of oxygen will undergo glycolysis (to produce pyruvate) and then go through the citric acid cycle and oxidative phosphorylation to produce a large amount of ATP, but in the absence of oxygen will convert pyruvate into lactate via anaerobic respiration to only produce a small amount of ATP. In cancer cells, regardless of the amount of oxygen present, pretty much all of their glucose metabolism goes down the glycolysis and anaerobic respiration route. This means that they are only producing a very small amount of ATP, therefor cancer cells need to up-regulate their GLUT1 (glucose transporter) to increase the amount of glucose they uptake.

- We can use immunohistochemistry staining and PET scans to image GLUT1 receptors in the body and therefore detect the presence of cancer cells.
- The most plausible hypothesis for why cancer cells benefit from glycolysis is that it yields raw material for making new nucleotides and amino acids etc (for rapid proliferation).
- Inhibition of hexokinase (first enzyme in glycolysis pathway) seems to be showing more potential than inhibition of GLUT1.

6. Genome Instability

Chemotherapy

- Non-targeted treatment leads to general side effects (bone marrow suppression, intestinal disorders, hair loss and impaired fertility), this limits the dose and frequency of administration

Therapeutic window – the range of doses at which a drug can be used at to obtain an effective treatment without being harmful. It may be possible to increase the therapeutic window if the right chemotherapy management drugs are used, eg. Management of nausea.

Chemotherapy resistance – Chemotherapy drugs are generally used in combination, to target many different areas of the tumour development, reduce toxicity to specific areas of the body and reduce the