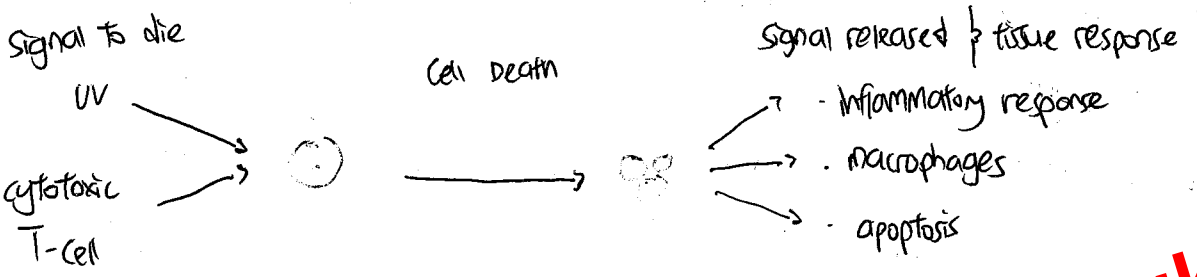
Apoptosis ... around ① million cells die every second

- Many disease involve defects in the normal regulation of cell death
  - ↳ degenerative diseases involve the loss of cells eg: neurons
  - ↳ immune system can kill our own cells eg: diabetes
  - ↳ cancer cells resistant to apoptosis
  - ↳ ischaemia / reperfusion results in the death of surrounding cells, leading to extensive damage, following stroke & heart attack

Dying cells release signals into their surroundings...



~ cells can die in many diff. ways, mode of cell death determine how the body responds

① Necrosis - causes inflammation
- swelling of cell
- loss of plasma membrane
- release of contents into surrounding tissue
- PM burst due to high pressure internally

② Apoptosis - does not cause inflammation
- damage to cell
- maintenance of plasma membrane integrity
- phagocytosed by macrophages
- no release of contents, but highly specific of certain chemicals

③ Autophagy
- maintenance of PM integrity
- organelles are broken down & released as nutrients
- helps cell stay alive a little longer when starved of nutrients by destroying organelles

Substrates for executioner caspases
1) myosin-light chain kinase, Rock-1
   ↳ cleavage activates the kinase
2) gelsolin, an enzyme

} affect changes in cytoskeleton causing membrane blebbing

Some BH3-proteins are regulated by growth factor signalling

↳ BH3 proteins can interact w pro-/anti-apoptotic Bcl-2 proteins

The balance between pro- and anti-apoptotic Bcl-2 proteins determine if a cell dies,

this can be altered in disease eg: cancer
- too much growth factor receptors → too much BH-3 that is anti-apoptotic
- too many pro-apoptotic Bcl-2 proteins, require more BH-3 interaction

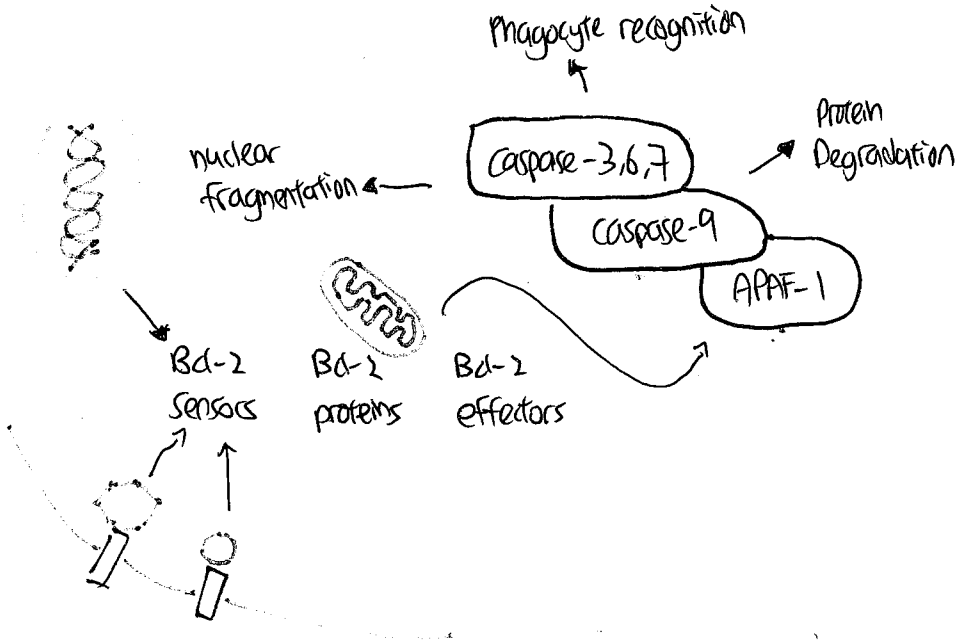
BH-3 proteins interact ~~with~~ with pro- and anti-apoptotic multi-domain Bcl-2 proteins via their BH3-domain

BH3-domain binding can either activate the pro-apoptotic activity of Bax & Bak or be

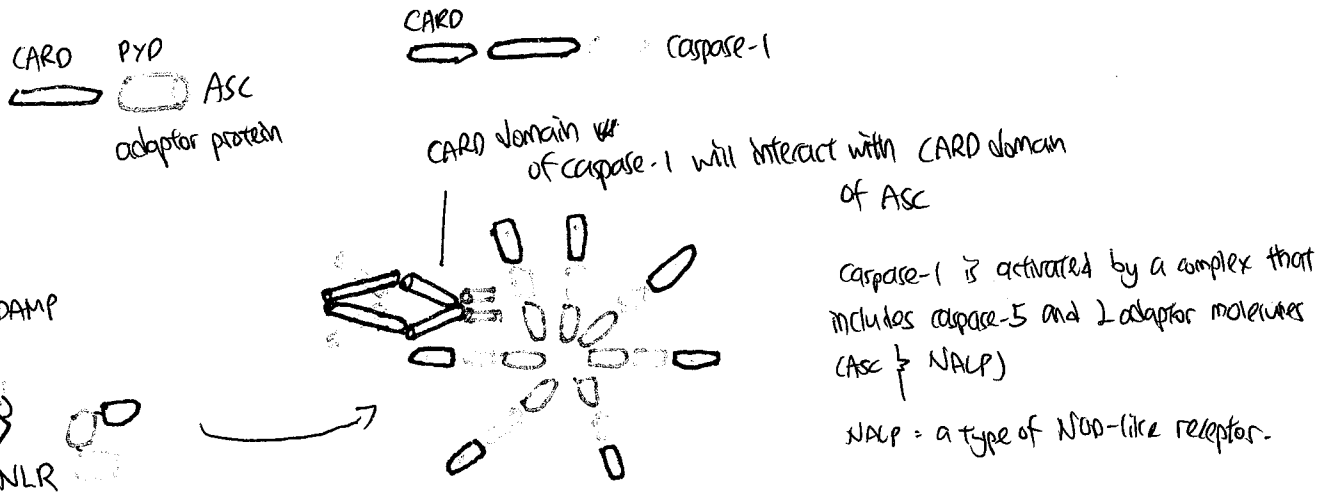
sequestered by binding the anti-apoptotic proteins like Bcl-XL and Bcl-2

Using structure to design compounds to mimic the BH3-domain

- NMR spectroscopy used to identify small molecules that bound the same region as BH3-domain
- Compounds joined to generate high affinity binding
- Structure based [illegible] to increase specificity



Caspase-1 is activated by the inflammosome



Caspase-1 is activated by a complex that includes caspase-5 and 2 adaptor molecules (ASC & NALP)

NALP = a type of NOD-like receptor.

Activated caspase-1 processes inflammatory cytokines as part of the innate immune response

↳ converts Pro-IL1 beta to IL1-beta
(inactive) (active)

Caspase-independent cell death =

1) mitochondria slowly loses function
2) Proteins like DNase, endonuclease G, Omi and apoptosis-inducing factor (AIF) released from intermembrane space

Necrotic cell death is passive, and release DAMPs

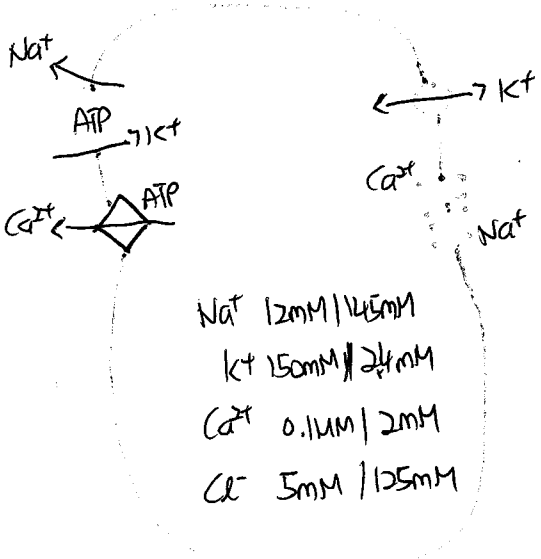
Morphological and physiological characteristics of necrosis

- organelles swell and plasma membrane ruptures
- this releases DAMPs, therefore can induce inflammation
- death is passive, not active as in apoptosis (no signalling pathway activated)

Necrosis can occur because of a variety of reasons

↳ ATP deprivation is a major clinical cause of necrosis

↳ ATP is required for the function of ion pumps in the plasma membrane. Loss of this function leads to loss of PM gradients, leading to swelling & rupture as H_2O comes in via osmosis



↳ Neurones and cardiac muscle are particularly energy hungry, and a major clinical sites for necrotic injury due to ischaemia

Acute Inflammation → could be directed against self antigens in autoimmune disease

- Rapid response to infection / injury
  - ↳ Hours to a few days
- Causes : microbial infections
  hypersensitivity reactions → Sensitive to metal
  physical
  chemical
  tissue necrosis

* inflammatory is not a disease, but a characteristic of diseases
** if inflammation does not occur, there is no repair process

tissue damage —(inflammation)→ removal of damaged tissue / pathogen → repair of damaged tissue

Macroscopic Appearance

- Suppurative (purulent) inflammation (pleural cavity) → lots of oedematous fluid, neutrophils → lots dead & dying cells / microbes
- fibrinous inflammation (acute pericarditis) → lots of fibrinogen deposited to form a thick layer of fibrin
- Pseudomembranous inflammation (colon)
- Serous inflammation eg: blisters → protein-rich fluid
- Catarrhal inflammation → hypersecretion of mucous by epithelial tissue
- Haemorrhagic inflammation → vascular damages eg: pancreatitis
- membranous inflammation – epithelial cells become covered by fibrin
- necrotising inflammation – rare
  high pressure in tissue preventing blood flow to it, may lead to gangrene

Characteristic Signs:

- Vascular events → due to ↑ blood flow
- Celsus : redness (rubor)
  : heat (calor)
  : swelling (tumour)
  : pain (dolor)
- Virchow : loss of function (added to Celsus' characteristics)
- Systemic events – fever
  – loss of appetite
  – lethargy
  – leukocytosis } acute phase proteins

Acute Inflammation

Aim of host : to deliver leukocytes and plasma proteins to injury / infection site for removal of necrotic tissue or pathogen

innate immune system: 1st line of defence

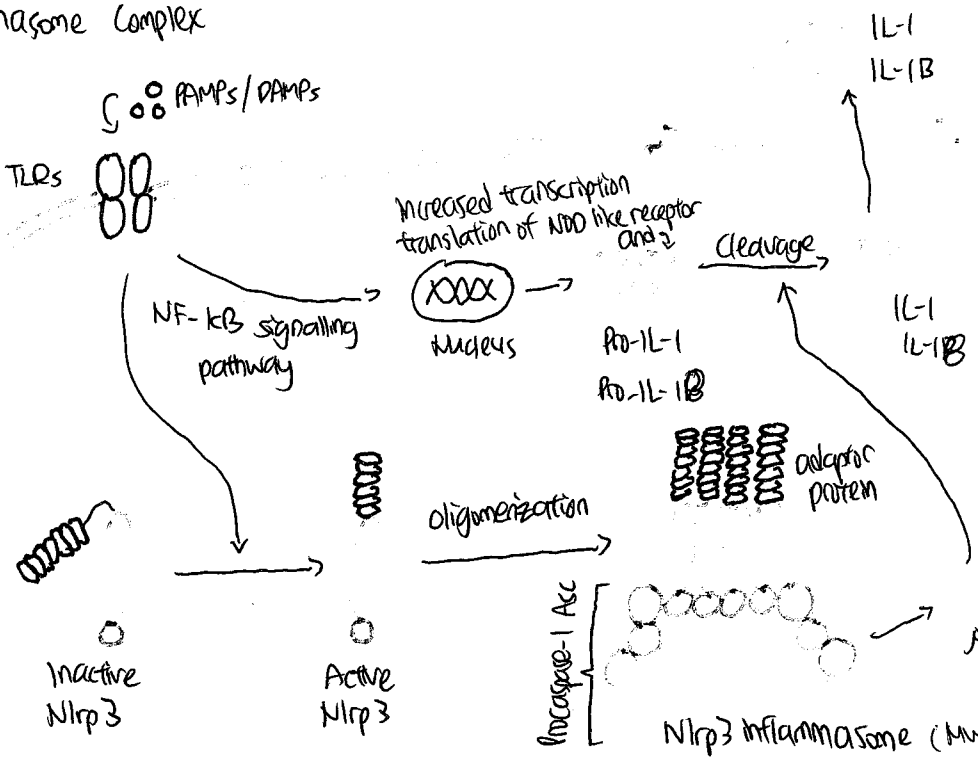
1) recognition of tissue damage / pathogen
   ↳ release of chemical mediators

2) recruitment of leukocytes and plasma proteins to the site
   ↳ increase in blood flow and capillary permeability

3) leukocytes and plasma proteins enter tissues
   ↳ release of chemical mediators
   ↳ phagocytosis
   ↳ elimination of pathogen / necrotic tissue

4) tissue repair

Inflammasome Complex

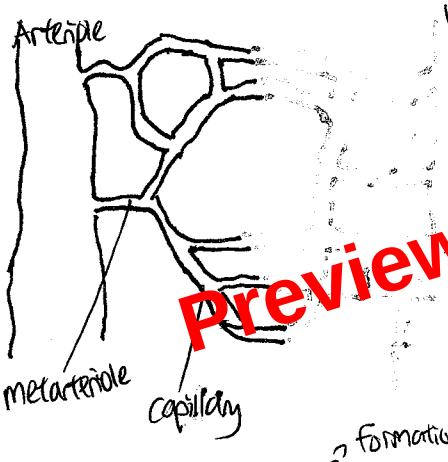


↝ chemokines are also produced to attract leukocytes to site of injury

↝ cryopyrin-associated periodic syndromes (CAPS) are characterized by mutation in Nlrp3 ↑, caspase-1 activation enhanced

Sensor protein: extracellular ATP, uric acid crystals, K^+ efflux, ROS, β-amyloid fibrils, PAMPs

Vascular stage: vasodilation



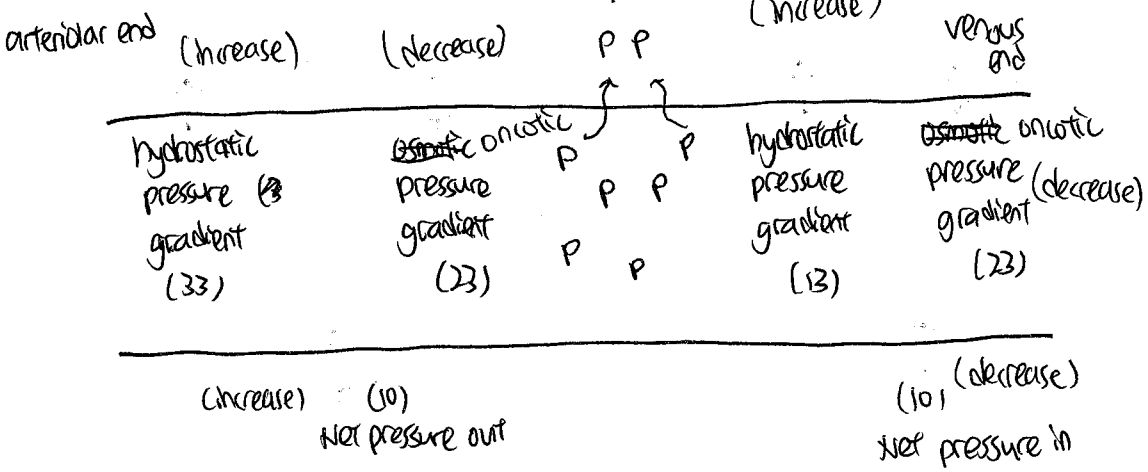
↝ Triple response to injury
- Red: vasodilation (increased blood flow)
- Wheal: [illegible] permeability
- Flare: axon reflex through activation of sensory nerve [nerve impulse run opposite direction] fibres → extra vasodilation → redness spread out

↝ VSM: induced by NO and histamine [vascular smooth muscle]

Vascular stage: vascular ~~leakage~~ permeability

↝ formation of early transudate (protein-poor filtrate of plasma) then exudate (protein-rich) into extracellular tissues

oncotic pressure gradient: generated by plasma protein
plasma protein moves out, thus ↓ oncotic pressure
endothelial cells contract, giving space for plasma protein to move out of vessel
P: plasma proteins



causes:
- chemical mediators
  eg: histamine, bradykinin, NO, complement component ~~C3a~~ C5a, LTB_4, PAF
- direct vascular trauma
- endothelial cell injury

- (changes) occur during inflammation
- PAF: platelet activating factor
- leukocyte bind to endothelial cells, damaging them, making it leaky

Inflammatory response	chemical mediators
vasodilation	- Histamine - Prostaglandins - NO
vascular permeability	- Histamine and serotonin / 5HT - C3a and C5a (indirect action by liberating vasoactive amines from mast cells) - Leukotrienes C_4, D_4, E_4
chemotaxis, leukocyte recruitment and activation	- TNF, IL-1 - chemokines - C3a and C5a - Leukotriene B_4
Fever	- IL-1, TNF - Prostaglandins
Pain	- Prostaglandins - Bradykinin
Tissue Damage	- lysosomal enzymes - ROS

Acute vs chronic inflammation

- weeks to months
- less marked systemic ~~effects~~ signs
- inflammation + tissue injury + repair

→ chronic inflammation can follow acute inflammation or develop on own, very often without any symptoms of ~~also~~ acute inflammation

→ chronic inflammation is usually primary

→ commonest variety of acute → chronic is suppurative

Causes

- immune-mediated eg: MS, IBD, rheumatoid arthritis
- persistent infection eg: tuberculosis, leprosy
- prolonged exposure to toxic agents
  - ↳ exogenous eg: abestos
  - ↳ endogenous eg: uric acid crystals (gout-causing)

Tissue Homeostasis

- During adult life, tissues are continuously replenished by the regulated proliferation and differentiation of somatic cells. This is called tissue homeostasis
- cancer is a ~~preventing~~ perverting of the rules governing homeostasis allowing the growth of new tissue (neoplasm) which is chaotically assembled

Hyperplasia (excessive proliferation of normal cells) → is NOT cancer (reversible) ← dependent on stimulus
↳ respond to stimulus (pathological/physiological)

metaplasia (replacement of one cell lineage w another) is NOT cancer (irreversible) ← dependent on chronic stimulus

Dysplasia (abnormal cytology / tissue architecture) is NOT cancer

10^{16} cell in a lifetime → less than one cell will give rise to a clinically significant tumour

cancer occur once assymetric stem cell division → progenitor daughter

↳ Barrett's oesophagus simple squamous → glandular

cancer
- a combination of hyperplasia, metaplasia, dysplasia
- a malignant neoplasm (neoplasia = new formation → neoplasm)
  - ↳ benign = mild dysplasia, restricted to anatomical site
  - ↳ malignant = highly dysplastic, cells invade adjacent tissue, generate 2° tumours [don't stay in the anatomical site] ← 80-90% cancer death
- often used synonymously w tumour
- a spectrum of disorders, often fatal, afflicting animals and man

* most tumours → benign

benign = non harmful
cancer = malignant neoplasm
malignant = harmful
tumour = mass / swelling

Cancer types

- epithelial → carcinoma (80%) — glandular epithelial and mucosa → adenocarcinoma; protective epithelia → squamous cell carcinoma
- bone, fat, muscle, connective tissue → sarcoma
- brain, nervous system → diverse eg: astrocytoma, neuroblastoma, Schwannoma
- blood, bone marrow, lymphoid system → leukaemia, lymphoma
- neural crest, neuroendocrine → diverse eg: melanoma

not all neoplasm are mass / swelling,
lymphoma, leukaemia → liquid tumours

* benign tumours can be associated w morbidity if they press on nerves, vessels, cause obstructions, secrete hormones

Nature of cancer - disease of faulty cells
- Tumours arise from normal tissue (not foreign bodies) and apparently from multiple cell types
- tumours are monoclonal in origin (arise from a single transformed cell)
- cancers seem to develop progressively (increasingly abnormal tissue homeostasis)

normal epithelium → hyperplastic → dysplastic → benign neoplasm → malignant → metastasis

cancers can spread in animals like an infectious disease

The Origins of Cancer

- Exposure to various agents is strongly associated w certain cancer-types in animal and man. This include:
  - ↳ chemical carcinogens eg: coal tars lung and skin cancer
  - ↳ physical carcinogens eg: UV and skin cancer
  - ↳ viruses eg: Epstein-Barr virus and Burkett's lymphoma
- Here the association between exposure and cancer is so great that the agents are considered aetiological factors (causes of cancer)
  - eg: polycyclic hydrocarbons present in coal tar which are carcinogenic
- other factors ↑ the risk of cancer but the effect is not absolute. Such risk factors identified by epidemiological studies include:
  - occupation (eg: abestos mining and mesothelioma)
  - reproductive history eg: age of 1st pregnancy, no. of pregnancies and breast cancer
  - diet eg: high-fat, red meat, processed food
  - lifestyle eg: smoking, drinking, sexual promiscuity, sun bathing
  - family history (cancer susceptibility can show Mendelian inheritance / polygenic inheritance)
- risk factors ↑ our exposure to aetiological agents and/or otherwise exacerbate disease progression

Environmental impact on Cancer Incidence

Ames test: test the mutagens ability to restore Salmonella's ability to synthesize histidine. (no ability to synthesize His)
If colonies formed → test chemical is mutagenic vice versa

chemical and physical carcinogens are often mutagenic

- X-rays and mustard compounds used as chemical weapons in WWI, both of which are carcinogenic, were discovered to be mutagens in Drosophila
- Bruce Ames developed a simple test for determining the mutagenic properties of chemical (The Ames test)
- There is close correlation between mutagenicity and carcinogenicity in rodents

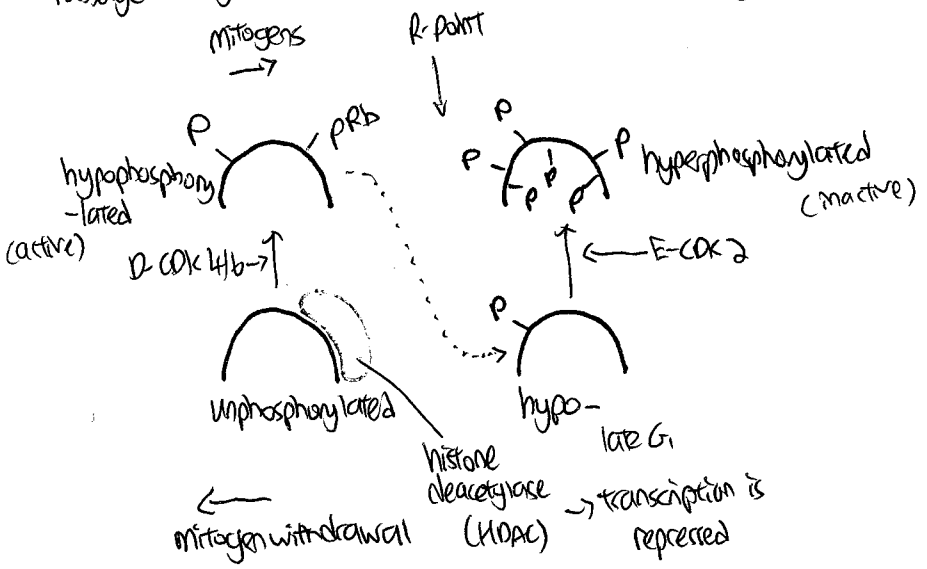
*CYP450 convert pro-carcinogens to carcinogen in liver

Known/suspected human tumour promoters → ↑ risk cancer by causing imbalance in body cells leading to excess growth (cancer)
[endogenous / exogenous GF] → stimulate growth of mutated cells

* Inflammation is also a major promoter

Aflatoxin: a potent carcinogen produced by Aspergillus mould that can grow in warm moist environment on fruits, vegetables & grains.

Passage through R point requires inactivation of Rb by phosphorylation



Rb is regulated by serial phosphorylation by cyclin-CDK that is initiated in G_1 (D-CDK4/6) and wiped after M phase by action of protein phosphatase 1 (PP1)

Rb is a family of 3 ~~pocket~~ pocket proteins (p107 and p130)

E2F is a family of 7 transcription factors

* securin binds to a protease, separase
APC targets securin for degradation, allowing separase to cleave proteins that hold sister chromatids together, initiating anaphase

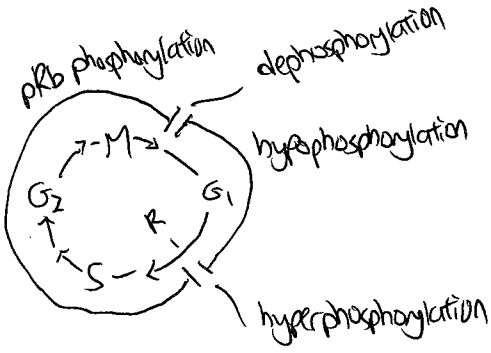
Herceptine: treatment for breast cancer

Some examples of cdk inhibitors: AT-7519
[all undergoing clinical trial] R-276-00
R 547 *

Rationale therapy for cancer based on suppressing mitogenic signalling

- anti-ErbB (HER2) receptor ~~mAbs~~ mAbs
- anti-HER1, HER2, HER4 tyrosine kinase inhibitors
- RAS ~~farnesylation~~ farnesyltransferase inhibitors
- RAF inhibitors
- MEK inhibitors
- mTOR inhibitors

Rationale therapy for cancer based on interfering with progression through cell cycle — CDK inhibitors: Pan-cdk, selective-cdk



phosphorylation regulates the interaction between Rb and transcription factor E2F. E2F/Rb complexes repress gene expression by recruiting HDAC. E2F alone can recruit HDAC and activate transcription.

Rb function to toggle between an activator/repressor of transcription

When Rb is hypophosphorylated, it can bind HDAC resulting in repression of transcription.

When Rb is hyperphosphorylated, it cannot bind HDAC
↓
transcription activation

Most cancers demonstrate deregulation of restriction point through a number of mutational & epigenetic mechanisms including amplification of cyclin D & deletion pRb

↳ cells will enter cell cycle more frequently

* the cycle WON'T BE faster

Cell death is important in many important processes
- around 1 million cells die in us every second
- occurs during development
- maintains homeostasis throughout life - tissue integrity

Detachment from a matrix induces apoptosis which must be overcome for metastasis to occur
↳ cells which lose contact w ECM undergo apoptosis (anoikis)

Bcl-2 family proteins control the mitochondrial (intrinsic) pathway of apoptosis
↳ Bcl-2 was first identified as an oncogene in human follicular B-cell lymphoma
- Reciprocal translocation between t (14:18)
- 14q32 IgH heavy chain JH region
- 18q21 Bcl 2 gene
- generated by defective VDJ rearrangements during B-cell development

Expression of Bcl-2 transgene was not in itself tumour promoting, BUT over-expression of Bcl-2 cooperates w overexpression of another oncogene - Myc
↓
B-cell lymphoma

BH-3 proteins promote Bax and Bak to directly permeabilise the outer mitochondrial membrane while Bcl-2, Bcl-XL prevents this → BH3 protein Bid, Bim, Bak, Bax multimerize to form the mitochondrial outer membran permeabilization complex (MOMP)

BH-3 proteins interact with pro- and anti- apoptotic multi-domain Bcl-2 proteins via their BH-3 domain

BH-3 domain binding can either activate the pro-apoptotic activity of Bax, Bak or inhibit the anti-apoptotic activity of Bcl-2 / Bcl-XL

direct activation model
displacement model
embedded together model

BH 3 proteins regulate multi-domain Bcl-2 proteins and respond to a number of 'abnormality' inputs
↳ various physiological } exogenous stress (including genotoxic chemotherapy } radiotherapy) operate through diff.
BH 3 proteins to antagonize different anti-apoptotic Bcl-2 family members

Regulation of BH-3 only proteins
↳ transcription
↳ sequestered in inactive forms
↳ proteolytic cleavage for activation

PI-3K-Akt is an essential module for survival factor signalling

PI-3K ~~is activated by~~
↳ activates Rac, a RhoGTPase

PI-3K → PDK1 → Akt1 → survival
↓
[protein-dependent kinase-1]

↳ mutation also causes JMML
* PTPN11 encodes ~~SHP~~ SHP2, a SH2 domain containing ~~PROTEIN~~ non-receptor tyrosine phosphatase
SHP2 is needed for the full activation of the RAS-RAF-MEK-ERK pathway
Mutation in PTPN11 → Noonan Syndrome
↳ dominant, gain-of-function allele
Mutation in SOS → Noonan syndrome
germline PTPN11 mutations → LEOPARD syndrome
↳ loss-of-function

Mutation in KRAS → Costello syndrome → predispose to rhabdomyosarcoma
at 12, 13 codon.

mutation in KRAS, BRAF, MEK1 } 2 → ~~craniofacio~~ cardio-facio-cutaneous syndrome

Drugs that block the signalling cascade — eg: Bevacizumab, Ranibizumab

- anti-VEGF antibodies that block VEGFR from binding VEGF.

bFGF: basic FGF

- IFN-α, also inhibits production of bFGF and VEGF, preventing these GF from starting the signalling cascade
- several synthetic drugs capable of interfering w VEGFR are being tested in cancer patients

VEGFR inhibitor: DC101, SU 5416

TNP-470: blocks endothelial cell proliferation

Drugs that target endothelial cells directly

- inhibit the growth of endothelial cells directly
- eg: endostatin, EMD 121974 (cilengitide) interferes with integrin $\alpha_v\beta_3$ binding by mimicking ECM peptide ligands, promoting destruction of proliferating endothelial cells

thalidomide: prevent angiogenesis in endothelial cells

: caused birth defects when taken by pregnant women (teratogenic)

Drugs that block ECM breakdown

- several synthetic drugs and naturally occuring molecules inhibit the activity of MMP are being tested eg: Marimistat, AG3340, BMS-275291 Batimastat

cancer metabolomics: the comprehensive analysis of full metabolite composition of cancer cells

cancer metabonomics: response of a system to a treatment

* combinatorial method: best option for angiogenic treatment

Glucose: a fuel and source of carbon for anabolic reactions

↳ changes to bioenergy production and intermediary metabolism in cancer cells

The 1st cancer hallmark - "altered metabolism" discovered by German, Otto Warburg in 1920s

Warburg effect - cancer cells displayed increased conversion of glucose into lactic acid (fermentation) even in the presence of O_2 (aerobic)

- more glucose is consumed and metabolites are diverted into biosynthetic reactions

Increased glucose uptake is exploited in FDG-PET imaging. FDG (fludeoxyglucose) is a radioactive glucose analogue and is >90% sensitive for metastasis detection → releases positron which can be detected

Glutamine is another major source of carbon for fuel and biosynthetic reactions exploited by cancer cells.

↳ glutaminolysis: a series of biochemical reaction by which the amino acid glutamine is degraded to glutamate, aspartate, CO_2, pyruvate, lactate, alanine and citrate (anaplerosis: metabolic pathway that replenish TCA intermediates)

↳ glutaminolysis is essential for energy production in cancer cells

↳ in tumour cells, citric acid cycle is truncated due to an inhibition of the enzyme, aconitase by high [ROS] generated by impaired oxidative phosphorylation

↳ tumour cells over express phosphate dependent glutaminase and NADP- dependent malate decarboxylase which in combination with the remaining reaction steps of citric acid cycle (from α-ketoglutarate to citrate) ↑ generates glutamate from glutamine

comprise a new energy producing pathway

Chronic inflammation is implicated in the pathogenesis of carcinoma

- inflammatory disease ↑ the risk of developing cancer (including bladder, cervical, gastric, intestinal, oesophageal, ovarian, prostate and thyroid cancer)
- NSAID ↓ risk of developing cancer such as colon & breast and reduce the mortality rate of these cancers
- Signalling pathways involved in the inflammation operate downstream of oncogenic mutations such as mutation in genes encoding RAS, Myc and RET
- inflammatory cells, chemokines and cytokines are present in the microenvironment of all tumours in experimental animal model and humans from the earliest stages of development
- the targeting of inflammatory mediators (chemokines and cytokines such as TNF-α and IL-1β), key transcription factors involved in inflammation eg: NF-kB and STAT3 or inflammatory cells decreases the incidence and spread of cancer
- adoptive transfer of inflammatory cells / overexpression of inflammatory cytokines promotes tumour development
- NSAIDS - commonly used as a preventative measure against familial cancers

An inflammatory infiltrate promotes tumourigenesis at multiple steps

Local invasion - an inflammatory ~~cytokine~~ infiltrate promotes angiogenesis and control ECM remodelling operating in paracrine loop. cytokines and growth factors produced by inflammatory cells and neoplastic cells induce [illegible] of genes associated w survival, invasion and migration

cytokines and GF are also involved in the intravasation of tumour cells into blood vessels and lymphatic spread

Distant metastasis - The autocrine and paracrine chemokine and cytokine mediated signalling promotes the survival of malignant cells in distant organs, attracting a tumour-promoting infiltrate and stimulate angiogenesis.

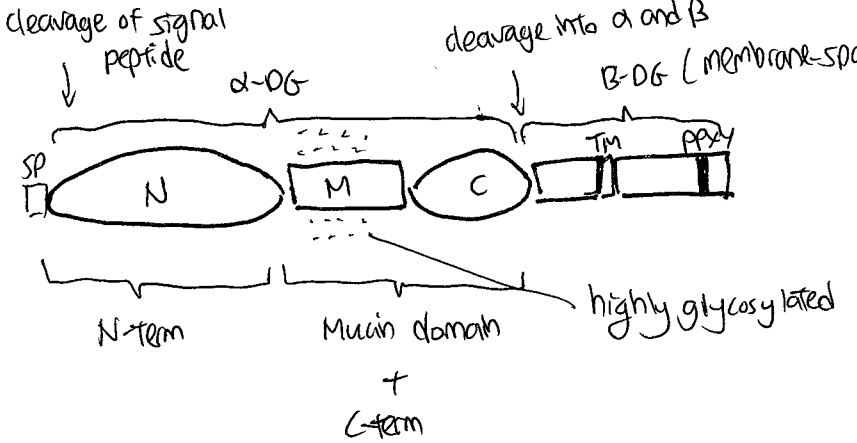
ROS produced by inflammatory cells is genotoxic

Dystroglycan – a TM laminin receptor independent of integrin

↳ ubiquitously expressed, but roles in tissues other than muscle are not clear

↳ mice with dystroglycan deletions are NOT viable

↳ NO known human mutations of dystroglycan, presumably because they are also lethal



SP: signal peptide

Core protein	30kDa	40kDa	41kDa
Glycosylated		156kDa	43kDa

Dystroglycan highlights how disease may result from mutations that are NOT in the immediate gene of interest

↳ α-dystroglycan is heavily glycosylated, and this varies in diff. tissues *glycosylation is tissue specific

The LG4 and LG5 domains of laminin α1 bind to glycosylated moieties on dystroglycan

↳ Removal of N-linked sugars does NOT affect laminin binding

↳ Removal of O-linked sugars inhibit laminin binding

Fukuyama congenital muscular dystrophy

- Autosomal recessive disorder found predominantly in Japan
- Severe muscle degeneration
- abnormalities in the brain
- caused by a 3kb transposon insertion into 3'UTR of fukutin gene, resulting in reduced mRNA expression
- Fukutin is involved in glycosylation of α-dystroglycan
- FCMD is associated with aberrant α-DG dly glycosylation
- This can be seen with antibodies that only recognize the glycosylated epitope

Dystroglycan

- Post translationally processed into α and β heterodimer
- C-terminal of β chain is transmembrane and cytoplasmic – attaches to dystrophin & other cytoskeletal components

→ Cow's milk: ~~Cow's~~ Controversial → may ~~lead to~~ be related to gut microflora

→ Wheat proteins: Controversial, may be regulated to gut microflora

→ Vitamin D: current opinion suggests that ~~it~~ it may be an important environmental factor that is required for the maintenance of self-tolerance and protection against autoimmunity

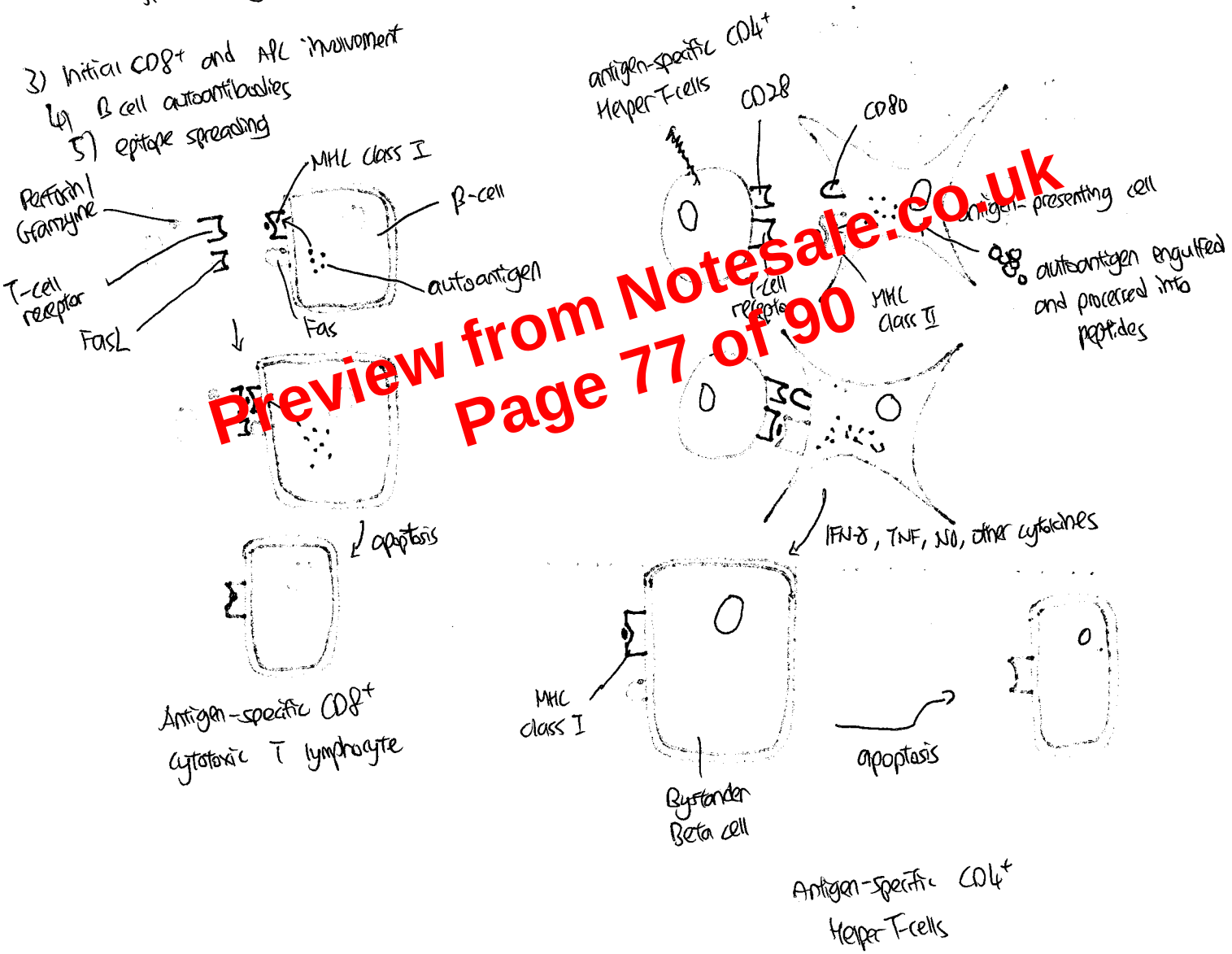
→ An unfortunate concurrence of genetic susceptibility and exposure to an environmental trigger which sets an individual up for developing diabetes

This triggered by inter-related events in and around β-cells

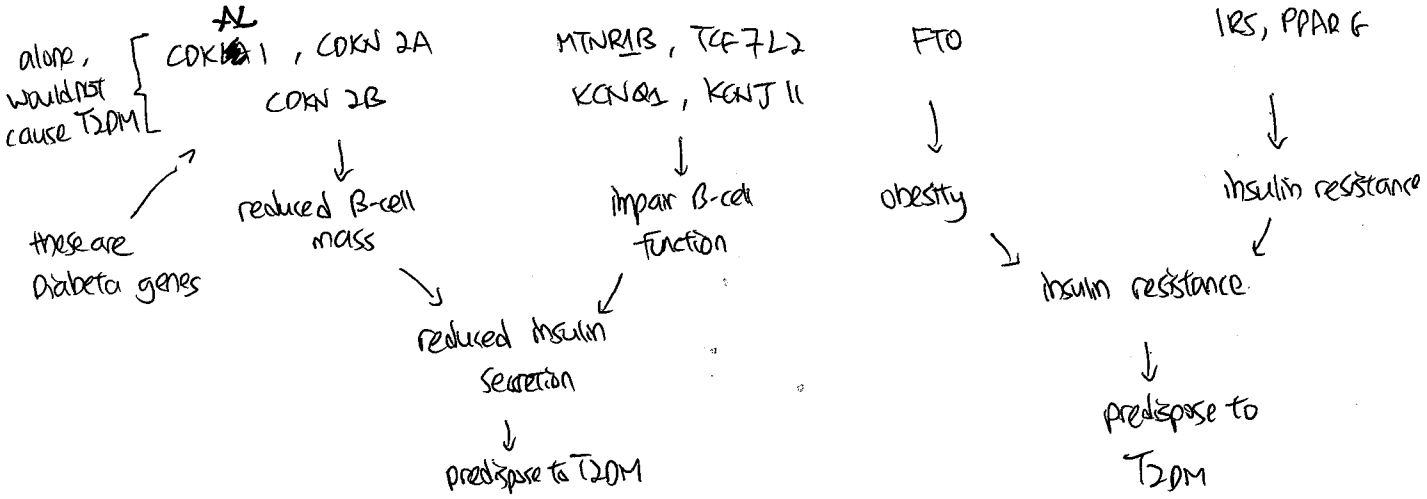
↓ upregulation of ~~cell~~ interferon (IFN-α) stimulates cytotoxic T-cells

↓ upregulation of MHC class I enabling cytotoxic T-cells to attack

3) Initial CD8+ and APC involvement
4) β cell autoantibodies
5) epitope spreading



B-cell dysfunction in diabetes: ↳ T2DM → poorly understood



The Insulin Receptor

- Heterotetramer of 2α and 2β chains { α chains: insulin binding; β chains: tyrosine kinase
- when stimulated w insulin, tyrosine kinase autophosphorylates and phosphorylates downstream targets (IRS 1-4 are major targets)

↓

docking of SH2 domain proteins for propagation of intracellular signalling → Activation of PI-3K and MAPK pathways

The Insulin Receptor and Signal Transduction

1) Ligand binding activates insulin receptor
2) tyrosine kinase phosphorylates receptor and IRS proteins to facilitate SH2 domain binding
3) Activation: PI-3 kinase pathway
   RAS / Ref / MAPK pathway
4) phosphorylation of downstream proteins to mediate changes in metabolism, gene expression and membrane transport including GLUT 4
5) consequences upon carbohydrate, lipid and protein utilisation & synthesis

GLUT 4: glucose transporter mediate uptake of glucose in insulin-sensitive cells

* in some patients w T2DM, insulin signalling, not the receptor is defective
  ↳ phosphorylation of serine, rather than tyrosine on IRS diminishes interactions with the PI-3K pathway