L14: ANTICANCER AGENTS

Cancer
- cancer cells (body's own cells)
  - uncontrolled proliferation
    - faster than parent cells, may be slower than other cells (RBC)
  - dedifferentiated state
    - back to SC (loss characteristic of parent cell), loss function, ✗ recog connection with other cells
  - invasive (intravasation)
    - 1° site → surrounding, loss recog of normal restraint, matrix-degrading E, surgery (remove surrounding cells too)
  - metastasis (extravasation)
    - 2° tumour distant from 1°, via lymphatics (or blood) → BM/lymph node/organs, hard to remove via surgery
- treatment
  - surgery (tumour, need early detection)
  - irradiation (damage DNA, adjunct to surgery)
  - chemotherapy (drug treatment)
- cause
  - mutation → inactivation of TSG
  - mutation → activation of protooncogenes → oncogenes

Tumour Suppressor Genes
- detect DNA damage
- p53
  - DNA damage → p53 activated → cell cycle arrest (arrest replication) → DNA repair / apoptosis (irreversible DNA damage)
  - gene most commonly mutated in >50% cancer
  - mutation (loss of function)
    - DNA damage unchecked → ✗ repair, ✗ apoptotic removal (irreversibly damaged DNA)
    - other mutations in cells accumulate
    - cancer

Protooncogenes
- cell growth & differentiation
- mutation → abnormal growth
- eg: GF, GFR (mutation→always on), members of GF signalling pathway (always activated), cell cycle transducer (faster)
- Ras
  - guanine nucleotide binding protein (GTPase)
    - Ras-GTP: on
    - Ras-GDP: off
  - component of many cell signalling pathway for proliferation
    - central point (converge on Ras → diverge)
  - mutation
    - 20-30% tumours
    - ↓ Ras inactivation (activated) → ↓ intrinsic GTPase, ↓ susceptibility to GTPase activating protein
    - Ras-GTP become predominant → always activated → cell divides w/o GF binding → proliferation

Chemotherapy
- exploit diff btw normal & tumour cells (hard to differentiate cancer cells)
  - growth rate (traditional)
  - other bio (contemporary)
- tumour growth = exponential = need near total removal for effective treatment
- log cell kill model
  - cell destruction = 1st order (constant fraction of cells killed)
  - 10^{12} tumour cells → kill 99.99% → 10^{8} tumour cells after 1 cycle of treatment → 10^{4} tumour cells after 2 cycles
  - side effects → need intermittent dosing → tumour regrowth, resistant cells (grow exponentially regardless of treatment)
- resting cells (tumour SC) resistant to treatment
- angiogenesis
  - growth is limited by blood supply to tumour
  - new target in therapy
- non-existent therapeutic window
  - treatment dose ≥ dose giving side effects
  - limit dose & therapy duration
  - although ✓ side effects → still give for survival
- if selective toxicity is via rapid cell division → toxicity will result from effects on other fast dividing cells
  - BM suppression → ✗ WBC → ↓immune system → susceptible to bac infection [immunocompromised]
  - impaired wound healing
  - hair loss
  - gut epi damage → nausea/vomit

Cytotoxic Anticancer Drugs

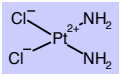
- kill cells: damage DNA, interfere DNA synthesis, mitosis
- mostly affect rapidly diving cells (going through cell cycle frequently)

(1) Alkylating Agents

- covalently bind to nucleophilic cell component (N7 & O6 of guanine)
- bifunctional: DNA cross linking (intrastrand → ✗ make new DNA)

Cisplatin

- discovered by chance while examining effect of electric current on bac (bac at platinum electrode → ↓DNA synthesis)
- 9 atoms, 2 leaving groups (Cl^- → covalent)
- bis-alkylation → intrastrand crosslink btw adjacent guanine → DNA denaturation
  trans isomer → inactive (still can bind DNA, ✗ form crosslink)



- treat
  - testicular & ovarian cancer
- side effects
  - nephrotoxic & neurotoxic
    - dose-limiting sensory neuropathy (irreversible, loss sensation)
  - severe nausea & vomit
    - cell release cytoplasmic content → detected in CTZ
    - ↑ nephrotoxicity
    - adjunct therapy: ondansetron ($5HT_3$ R antagonist)
  - BM suppression

(2) Antimetabolites

- inhibit DNA synthesis pathway
- folate —dihydrofolate reductase→ dihydrofolate —dihydrofolate reductase→ tetrahydrofolate ⤵
  2'-deoxy uridylate (dUMP) —thymidylate synthetase→ 2'-deoxy thymidylate (dTMP)
  ↳ for DNA synthesis

Methotrexate

- thymineless death (apoptosis)
- ↑ affinity for dihydrofolate reductase (DHFR) than folate (natural subs)
  inhibit DHFR → inhibit tetrahydrofolate (FH_4) (cofactor) → inhibit thymidylate production (dTMP) → inhibit DNA synthesis
- ↓ lipid solubility → ✗ cross BBB
- resistance (use ↑dose + folate b/c diff uptake of folate in cells)
- side effects
  - BM depression
  - gut epi damage (✗ absorb nutrient)

5-Fluorouracil

- prodrug → Pi into 5-fluorodeoxyuridine monophosphate (FdUMP)
  - false subs for thymidylate synthetase → inhibit thymidylate → inhibit DNA synthesis
- folate —dihydrofolate reductase→ dihydrofolate —dihydrofolate reductase→ tetrahydrofolate ⤵
  5-fluoro-2'-deoxyuridylate (FdUMP) —thymidylate synthetase→
- administered with folate
  - inhibited E is a complex with FdUMP & cofactor from folate → ↑ toxicity
- side effects
  - BM toxicity
  - gut damage

(3) Anthracyclines

Doxorubicin

- planar → binds DNA by intercalation (slide btw bp)
- topoisomerase II
  - DNA unwinding/rewinding to relieve supercoiling
  - nick/rejoin DNA
- inhibit topoisomerase II → nicked DNA → apoptosis
- side effects
  - cardiotoxic
    - produce free radicals → damage cardiac tissue
    - ↓ by co-administration of dexrazoxane (Fe chelator → ↓Fe^{2+} mediated free radical production)

(4) Microtubules Inhibitor

- microtubules
  - hollow cylindrical fibres of α & β-tubulin
  - separate diploid chr pair
  - dynamic instability: length depends on relative rate of polymerisation vs depolymerisation (GTP→GDP dependent)

Paclitaxel

- from yew tree bark
- promote microtubules polymerisation & inhibit depolymerisation
  - prevent spindle formation
  - cells ✗ separate → ✗ form chr
  - affect cells that divide more often
- side effects
  - myelo/BM suppression
  - neurotoxicity (microtubules — axonal transport)
  - nausea, vomit, hair loss

L15: ANTI-CANCER AGENTS

Drugs that exploit other aspects of tumour bio
- selective toxicity based on rapid cell division = flawed = dose-limiting side effect, kill WBC too
- drugs should be more selective, ↓side effect, desired dose can be administered

(1) Hormones
- used when tumour growth is dependant on hormones
- oestrogen
  - functional antagonist
  - androgen-dependent prostatic tumour
- anti-oestrogen
  - oestrogen R antagonist (remove tissue sample → check whether tissues exp oestrogen R → if no → useless)
  - tamoxifen
  - oestrogen-dependent breast cancer

Angiogenesis
- growth of new blood vessels from pre-existing vessels
- tightly controlled, normal phys (late embryonic dvlp, menstrual cycle, wound healing)
- angiogenic switch
  - small localised tumour → signalling mol to nearby BV → angiogenesis → solid tumour grow & spread (red BV surround it)
  - if ✗ angiogenesis → small, ✗ grow larger
- multistep process
  - degradation of basement membrane by matrix metalloproteinase
  - endo cell → migration & proliferation
  - form new matrix
  - stabilised by pericytes
- endothelial cell proliferation
  - controlled by many GF
  - VEGF (vascular endo GF)
    - tumour in angiogenic switch produce VEGF → endo cell proliferation → angiogenesis
- for growth & spread of solid tumour
  require new blood endo cells (VEGF control endo cell proliferation)
  VEGF inhibitor → novel anti-tumour agent

Metastasis
- invasive
- cancer cells invade surrounding tissue/vessels → transported via circ system to distant site → usually go to lymphatic vessels (↓p, ✗ p resistant) → reinvade & grow at new location
- surgical removal is useless (✗ remove 2° tumour)

(2) Anti-Angiogenesis Agent
- 5 VEGFs
  - disulphide-linked dimer
  - solvent exposed loops
    - form 2 poles of R binding
    - mimetics = VEGF antagonist (prevent VEGF binding to its R)
- VEGFR
  - tyrosine kinase R (dimerisation → proliferation)
  - VEGFR 2 & 3 → selectivity
- VEGF inhibitors
  - inhibit ligands (anti-VEGF mAb)
  - inhibit R (TK inhibitor, competitive R antagonist)

Ligands	Receptors	Location
	VEGFR-1	
placental GF		
VEGF-A	VEGFR-2 (!)	blood vessels
VEGF-B		
VEGF-C	VEGFR-3 (!)	lymphatic vessels
VEGF-D		

Bevacizumab
- monoclonal antibody to VEGF
  - binds VEGF-A → prevent VEGF-A binding to R
  - stop endo cell proliferation
- MOA
  - induce tumour hypoxia/starvation
  - ↓ VEGF-mediated increase in vascular permeability → ↓ interstitial p → improve drug delivery
- + cytotoxic drugs → best clinical effect
  - metastatic colon cancer
  - unethical to use placebo in cancer → should use current cytotoxic vs current cytotoxic + new drug
- side effects
  - proteinuria, impair wound healing
  - HP, thrombosis/bleeding
  - ✗ effect on WBC → ✗ cytotoxic

(3) Signalling Pathway Inhibitor

VEGF RTK Inhibitor
- small mol that bind active site of TK domain
- sunitinib
  - inhibit VEGF-1, VEGF-2, PDGFR
- sorafenib
  - inhibit VEGF-1, VEGF-2, PDGFR, B-RAF
- lack selectivity (inhibit multiple TK) → good for renal cell carcinoma
- VEGFR-2 bioassay
  - monomeric monocyclic peptide
  - preclinical dvlp (Vegenics)

Chronic Myeloid Leukaemia (CML)
- 15% adult leukaemia
- only 1 mutation
- BCR-ABL kinase constitutively activated
  - chromosomal translocation → philadelphia chr
  - fusion of 2 genes = BCR & ABL (ABL is a tightly reg TK)
  - BCR-ABL essential for leukaemic cell survival
- poor prognosis (6yrs)

Imatinib Mesylate
- BCR-ABL kinase inhibitor (small mol)
  - bind kinase domain → stabilise protein in closed/inactive conf
- inhibit growth of BCR-ABL kinase exp cells *in vitro*
  suppressed growth of BCR-ABL exp human tumours in mice
  only for some patients
- phase I
  - haematologic response: normalisation of blood count in 95% patients
  - cytogenetic response: ↓ philadelphia chr-positive cells
- phase III
  - better haematologic & cytogenetic than standard cytotoxic treatment
- clinical use
  - NOT cure, slows CML progression
  - resistance → ✗ cure
- resistance
  - BCR-ABL mutation → prevent closed conf → imatinib ✗ bind
  - new classes of TK inhibitor
    - bind irrespective of open/closed conf
    - ↑ affinity against WT
    - bind imatinib-resistant mutants
- side effects
  - nausea, vomit, muscle cramp
  - liver toxicity, fluid retention

Drug Resistance
- 1° = when drug first given
  2° = dvlp during treatment

  hard to know 1° or 2°
- ↑ dose for same killing effect → ↑ side effect → limit effectiveness of chemotherapy
- tumour cells dvlp resistance
  - ↑ cell number
  - rapid growth rate
  - ↑ mutation rate (loss of function of TSG)
  - drug treatment inherently selects for resistant cells
- our WBC has stable genome & ✗ mutation → ✗ resistant

Drug Resistance MOA

↓ intracell accumulation	↑ P-glycoprotein (drug transport protein)	- in membrane - part of BBB (remove toxin), protect against envm toxins - active efflux pump (broad specificity) • pump out >1 type of drug → multi-drug resistance - resistance ✗ depends on MOA, based on ability to come out of cells
↓ uptake by cell	methotrexate	↓ exp of folate carrier
↓ activation by cell	5-fluorouracil	Pi → inactivation
↑ inactivation by cell	antimetabolites	deaminase → inactivation
insensitivity to apoptosis	loss p53 (tumour suppressor function)	cells with damaged DNA → ✗ apoptosis
	leukaemia, lymphoma, testicular cancer	WT p53 = responsive to chemotherapy chemo → p53 recog damaged DNA → apoptosis (cytotoxic)
	pancreas, lung, colon cancer	mutant p53 → poor killing = poor response to chemotherapy

Cancer Chemotherapy
- cytotoxic drugs → main therapy
- newer agents
  - more selective
  - less dose-limiting side effect
- still ✓ resistance