Veterinary Bioscience: Cells to Systems

VETS90121 / VETS30029

Neoplasia 2

Lecture 36

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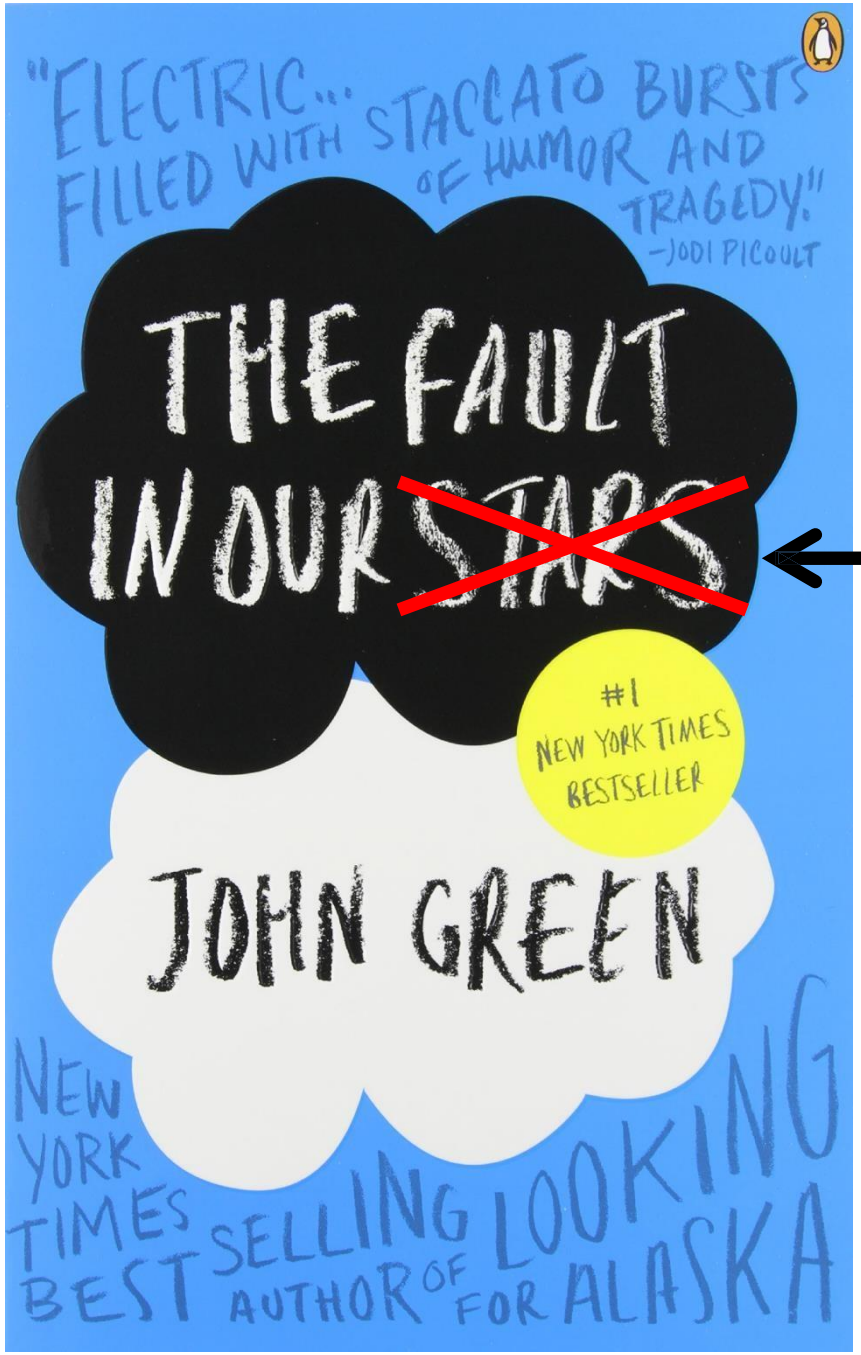
FACULTY OF VETERINARY & AGRICULTURAL SCIENCES

Learning objectives

- By the end of this lecture you should be able to:
  - List and define the types of genes involved in neoplastic transformation
  - Explain the process of neoplastic transformation
  - Describe the hallmarks of neoplasia
  - List the major causes of neoplasia and explain their mechanism of action

Why does neoplasia develop?


"ELECTRIC... FILLED WITH STACCATO BURSTS OF HUMOR AND TRAGEDY!"
—JODI PICOULT
THE FAULT IN OUR STARS
#1 NEW YORK TIMES BESTSELLER
JOHN GREEN
NEW YORK TIMES BEST SELLING AUTHOR OF LOOKING FOR ALASKA

← Genome

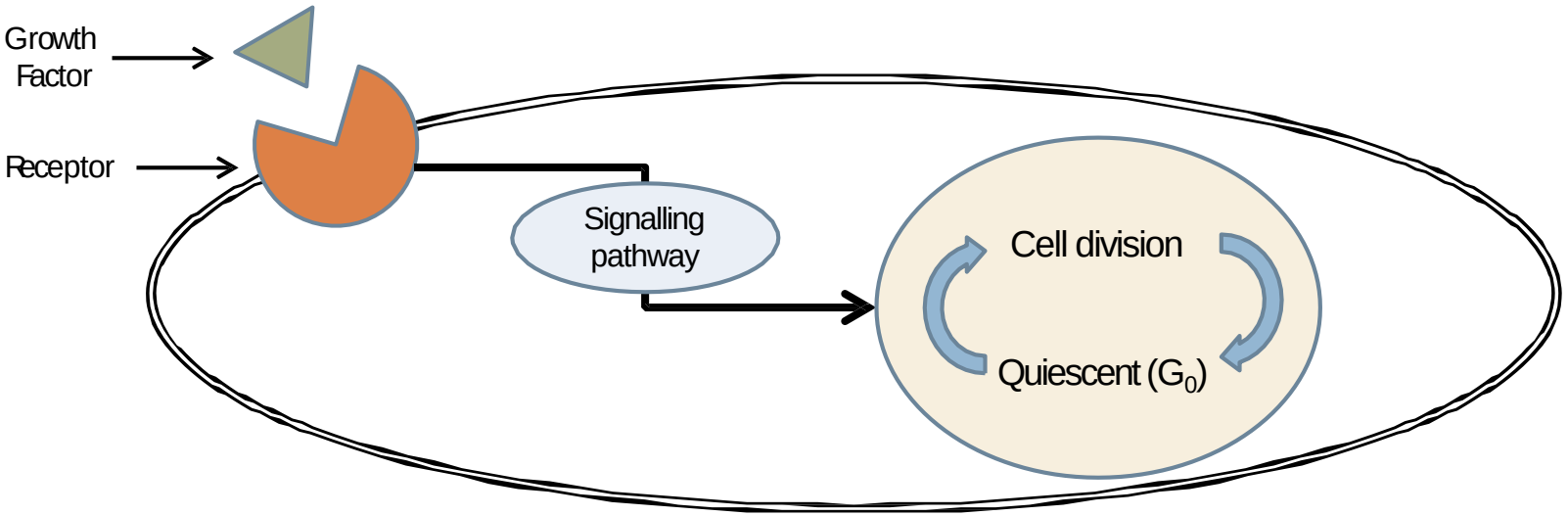
Development of neoplasia

- Irreversible, non-lethal genetic change causing unregulated cell growth

- Neoplastic transformation is generally initiated by alterations in the following gene types
  1. Oncogenes
  2. Tumour suppressor genes
  3. Genes that regulate DNA repair
  4. Genes the regulate apoptosis

1. Oncogenes

- Overactive versions of normal genes that promote cell growth and proliferation (proto-oncogenes)
  - Activation may result in:
    - ✓ Increased gene product expression
    - ✓ Increased gene product activity
    - ✓ Decreased gene product clearance/metabolism
  - Activation only requires mutation of one gene copy ("one hit").
  - Some types of proto-oncogenes include:
    - ✓ Growth factors and growth factor receptors (eg. EGFR – epidermal growth factor receptor)



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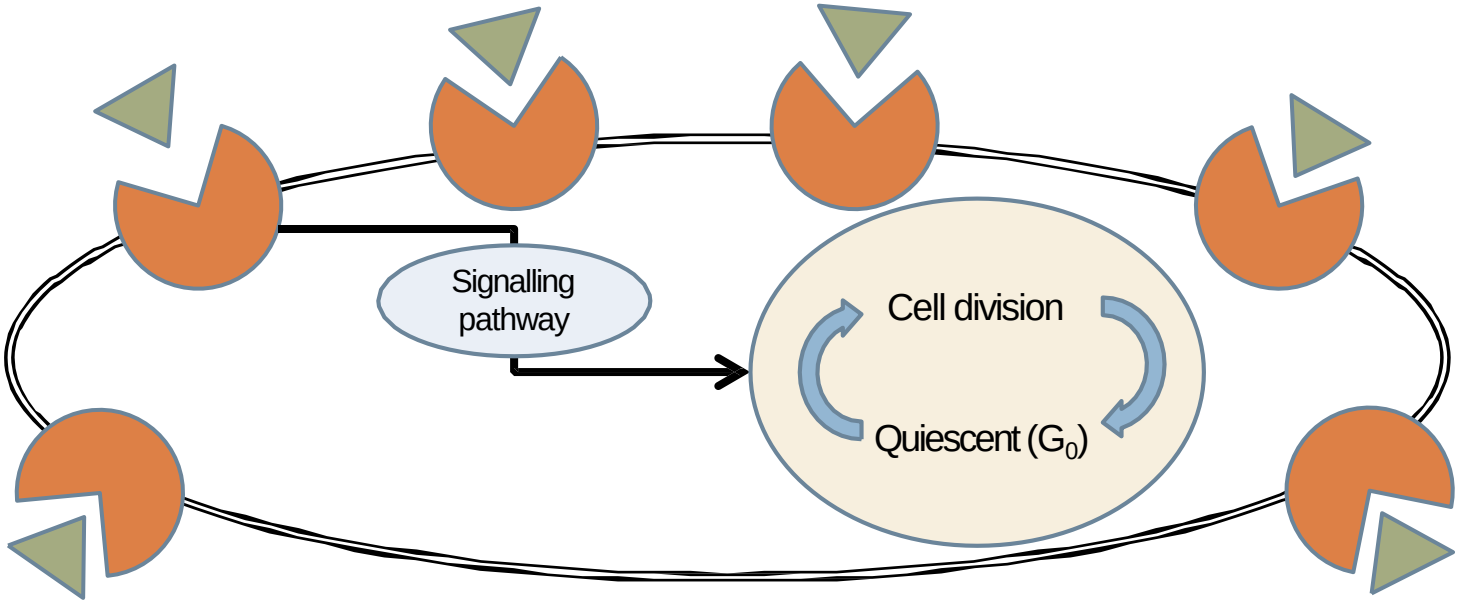

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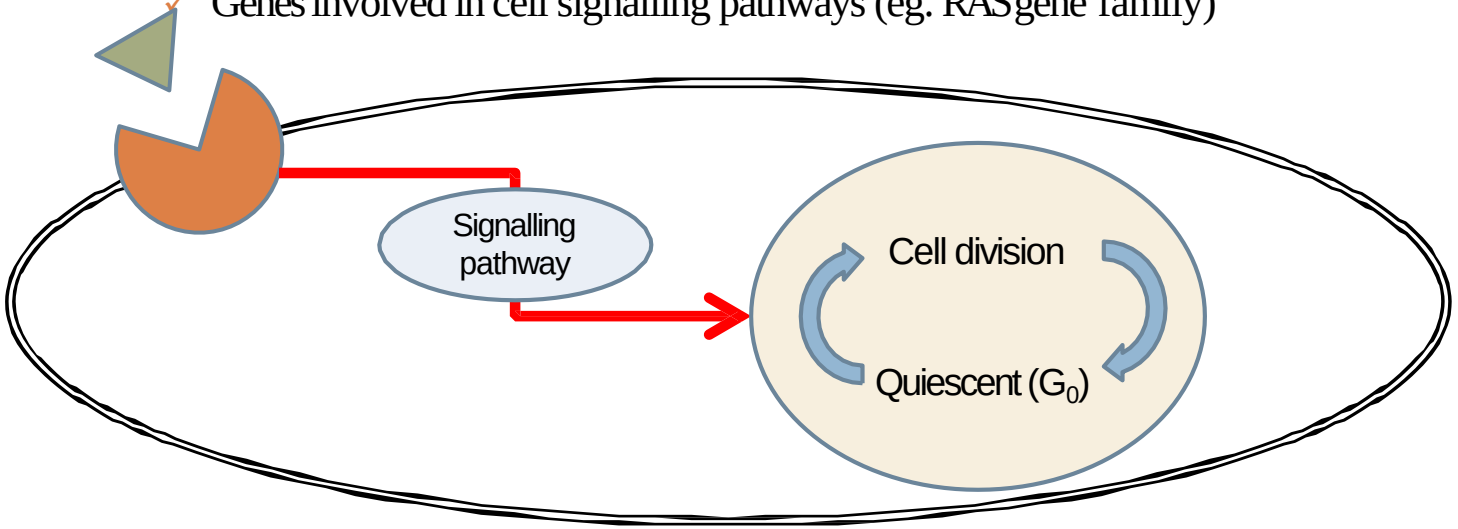
    - ✓ Genes involved in cell signalling pathways (eg. RAS gene family)



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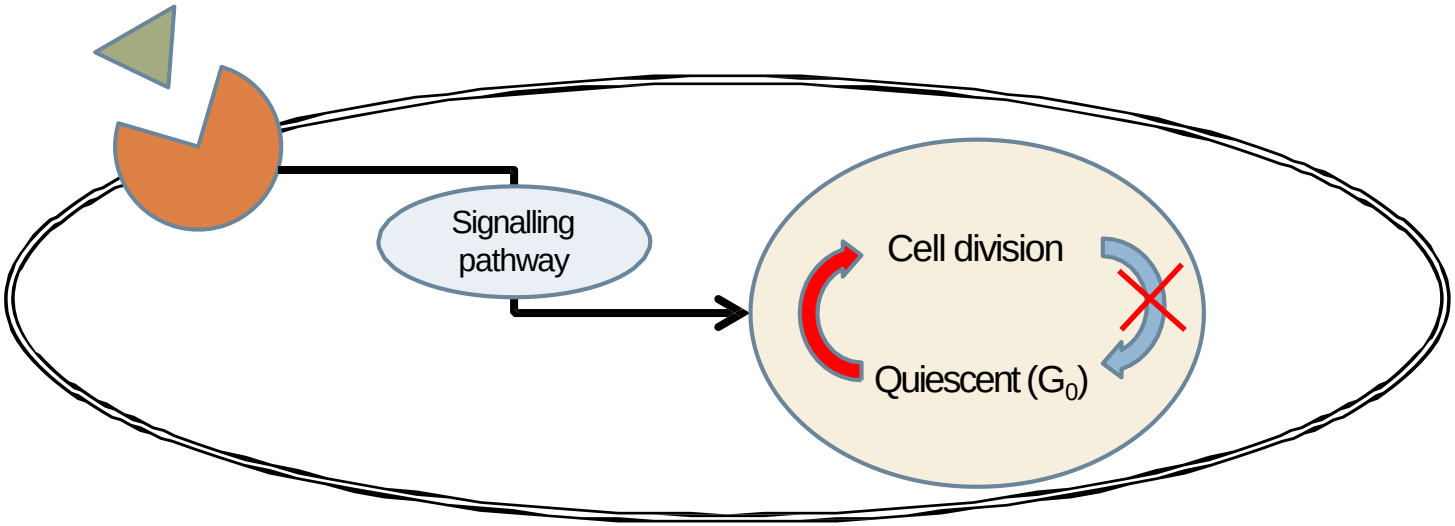
    - ✓ Genes promoting cell growth and division (eg. cyclin D1 - regulates movement through stages of division)



2. Tumour suppressor genes

- Genes which regulate cell growth ("governing genes")
  - Retinoblastoma (Rb) gene
    - ✓ Inhibits cell division until appropriate time
- Genes which monitor for pre-neoplastic changes in cells ("guardian genes")
  - Tumour Protein p53
    - ✓ Senses DNA damage and signals cell cycle arrest or apoptosis

Vet Pathol 40:237–248 (2003)

Clinical and Pathologic Relevance of p53 Index in Canine Osseous Tumors

P. Loukopoulos, J. R. Thornton, and W. F. Robinson

School of Veterinary Science, The University of Queensland, Brisbane, Queensland, Australia

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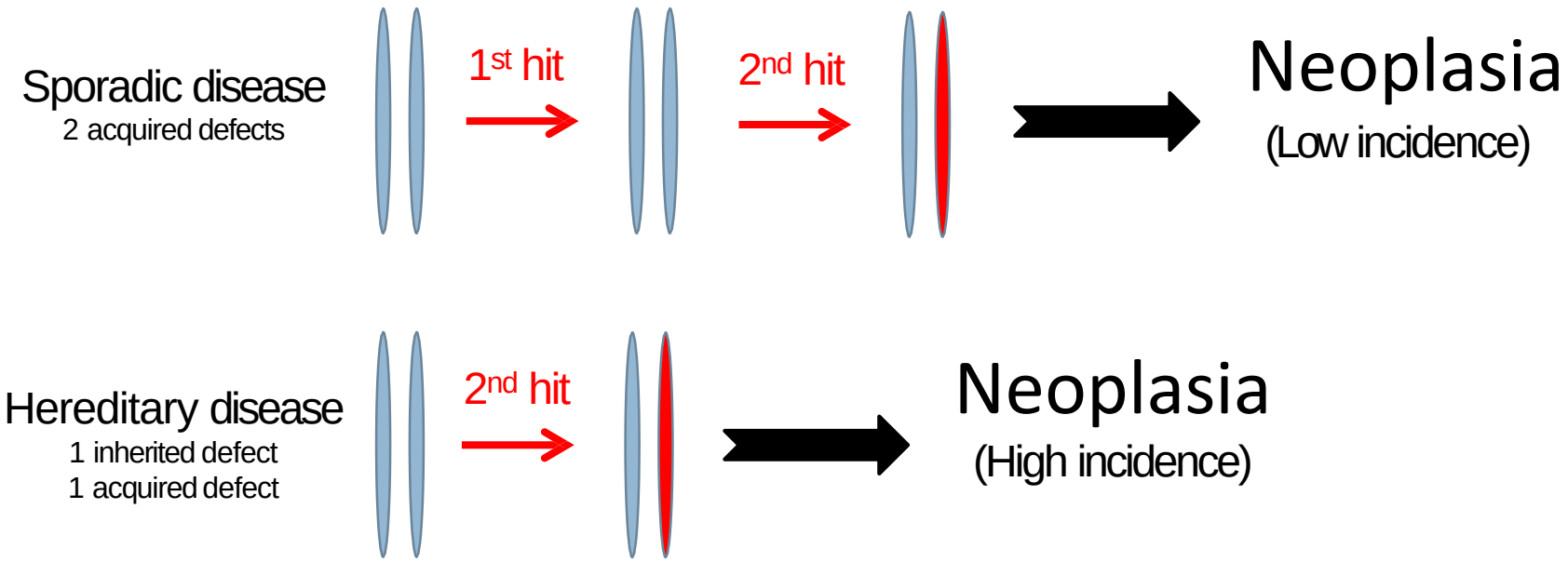
  - Tumour Protein p53
    - ✓ Senses DNA damage and signals cell cycle arrest or apoptosis
- Inactivation leads to loss of regulation of cell proliferation and neoplasia
  - Both gene copies need to be damaged ("two-hit")

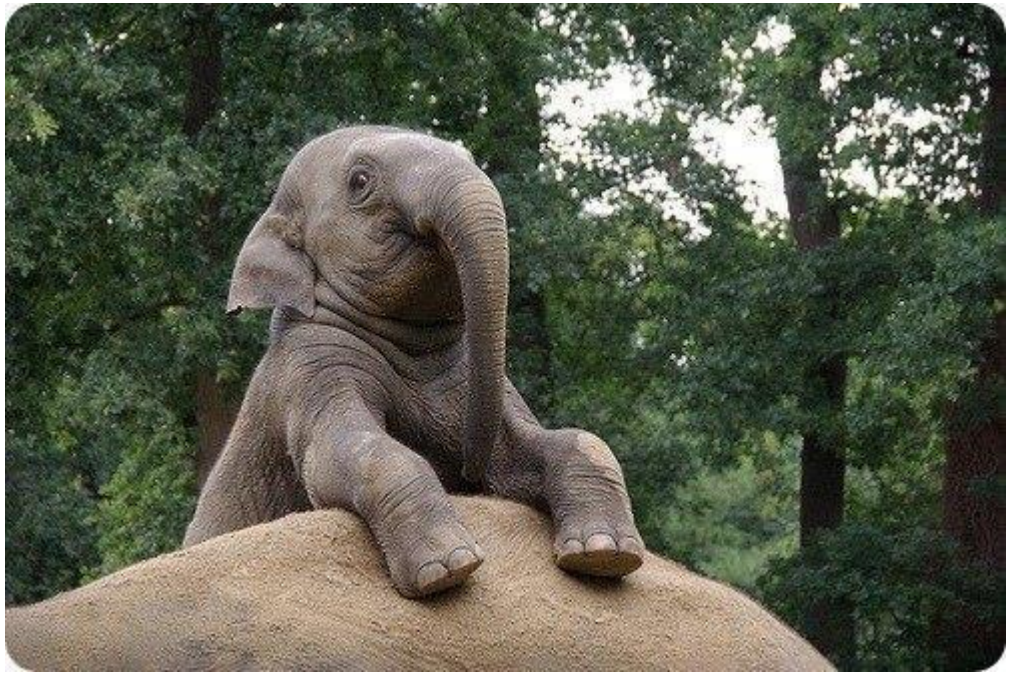


Why don't elephants get cancer?

- Large, long-lived animals
  - Have lots of cells with lots of time to become neoplastic
  - Incidence of neoplasia should be high, but is not

Why don't elephants get cancer?

- Genomic analysis
  - Humans genome contains 1 p53 gene pair
  - Elephant genome contains 20 p53 gene pairs
    - All 40 copies need to be inactivated to promote neoplasia!



Abegglan et al. JAMA, 2015; 314(17):1850-1860.

3. DNA repair genes

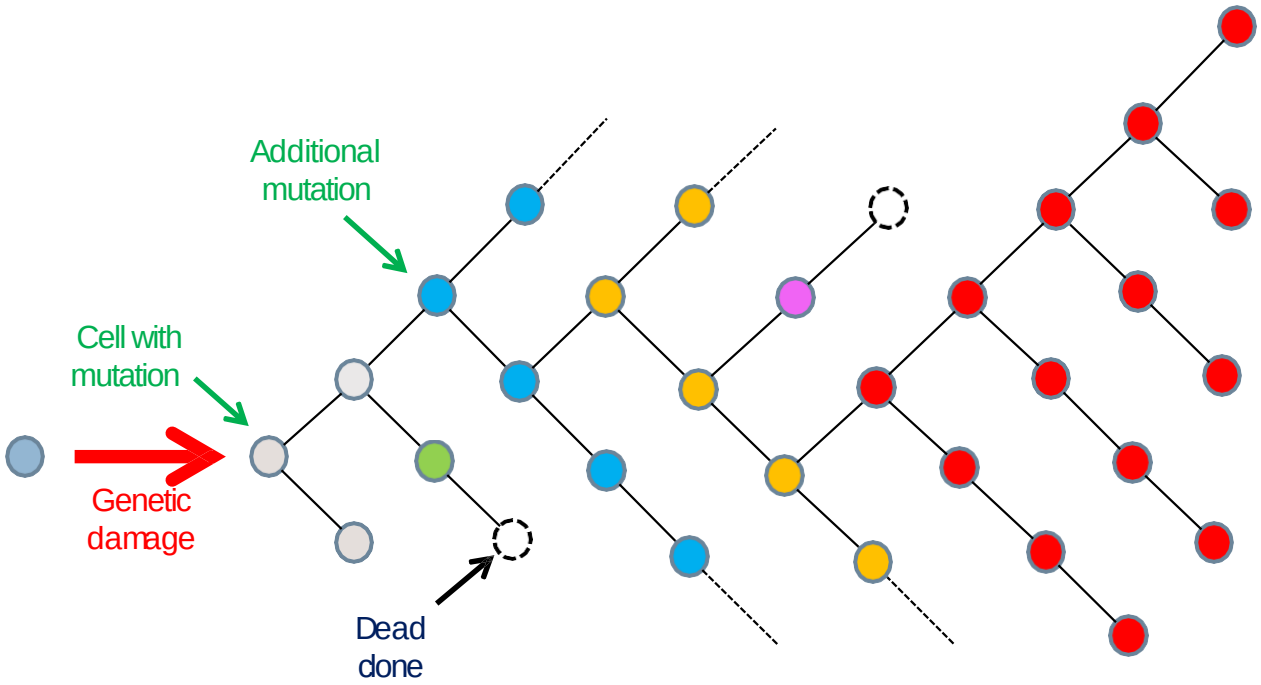
- Genetic defects accumulate over time
  - Spontaneously
  - Secondary to carcinogens
- DNA damage normally sensed and repaired by variety of repair genes
- Defective or inactivated DNA repair genes result in poor maintenance of DNA integrity, accumulation of mutations and promotion of oncogenesis

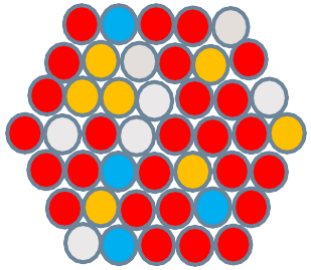
4. Genes that regulate apoptosis

- Apoptosis is the mechanism used to eliminate rogue cells that are on the path to neoplasia
  - Mutations impairing apoptotic pathway allow damaged cells to survive and continue progression to neoplasia

Development of neoplasia

- Multistep phenomenon with accumulation of genetic defects
  - Neoplastic evolution: selective growth of best-adapted cells





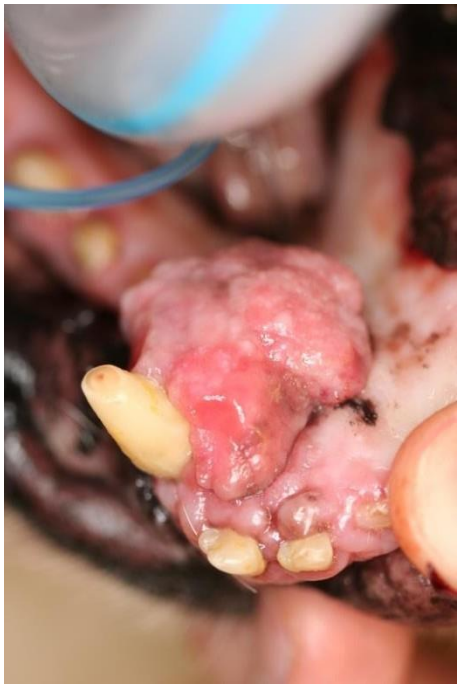
Neoplasms are heterogeneous masses composed of many clones with slightly different mutations

Additional mutations and proliferation of best-adapted clones

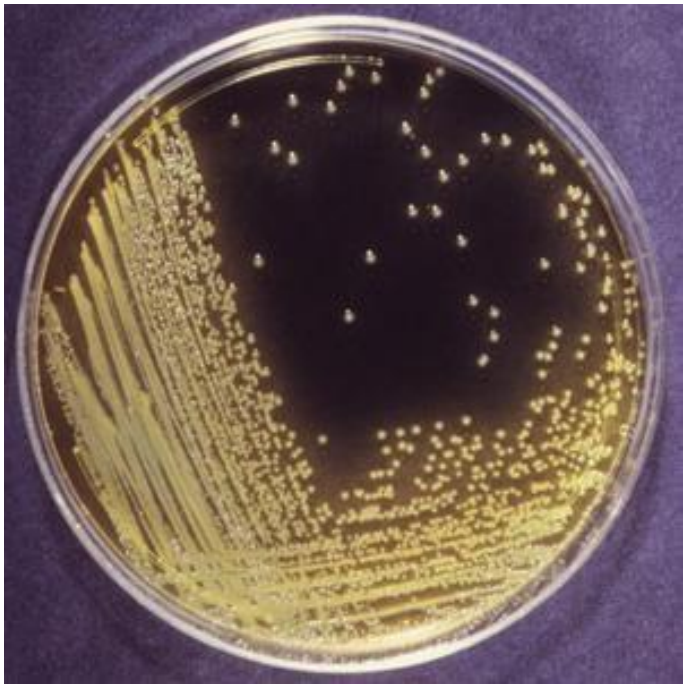
Pre-neoplastic ---------- Neoplasia

Antineoplastic drug resistance

Chemotherapeutic drug-resistant neoplasm



Antibiotic-resistant bacteria



Both involve the same process – selective growth of resistant clones

“Hallmarks of neoplasia”

Successful neoplasms display the following attributes:

1. Self-sufficiency in growth signalling
2. Insensitivity to anti-growth signals
3. Evasion of apoptosis
4. Limitless proliferation
5. Sustained angiogenesis
6. Altered cellular metabolism
7. Evasion of the immune system
8. Tissue invasion and metastasis*

* Features of malignancy

1. Self-sufficiency in growth signalling

- Normal tissues require external signals (growth factors) to proliferate
- Neoplastic cells promote their own proliferation via:
  - Oncogene activation
    - ✓ Reduces or eliminates requirement for growth signals
  - Persistent growth factor secretion
    - ✓ Self-secretion (autocrine signalling)
    - ✓ Inducing secretion by stroma

2. Insensitivity to growth inhibitory signals

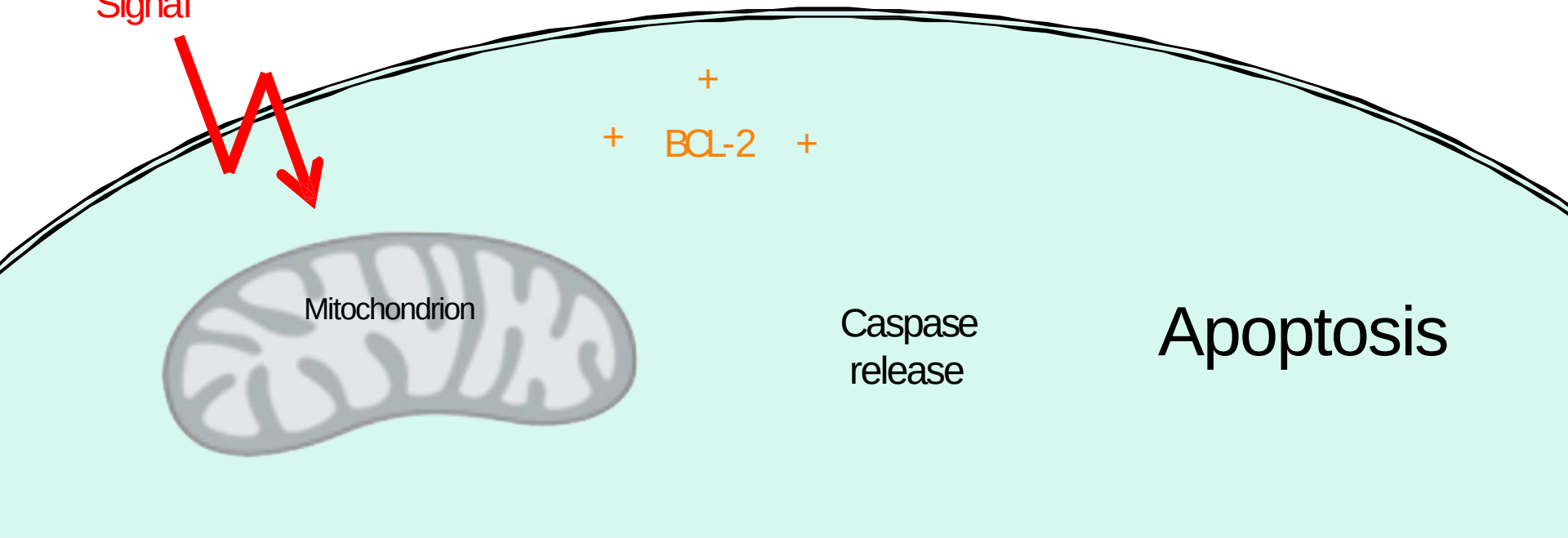
Lack ability to sense when things go bad
(Tumour suppressor genes inactivated)

Don't care about personal space
(Loss of cell-cell contact inhibition)

Ignore warnings to stop
(Insensitive to inhibitory cytokines eg.TGF-β)

3. Evasion of apoptosis

- Apoptosis is the major mechanism for eliminating damaged cells before they become fully neoplastic
- Neoplastic cells are resistant to apoptotic signaling
  - ✓ BCL-2 proteins suppress the intrinsic (mitochondrial) apoptotic pathway
  - ✓ Overexpression of BCL-2 blocks initiation of apoptosis



4. Limitless proliferation

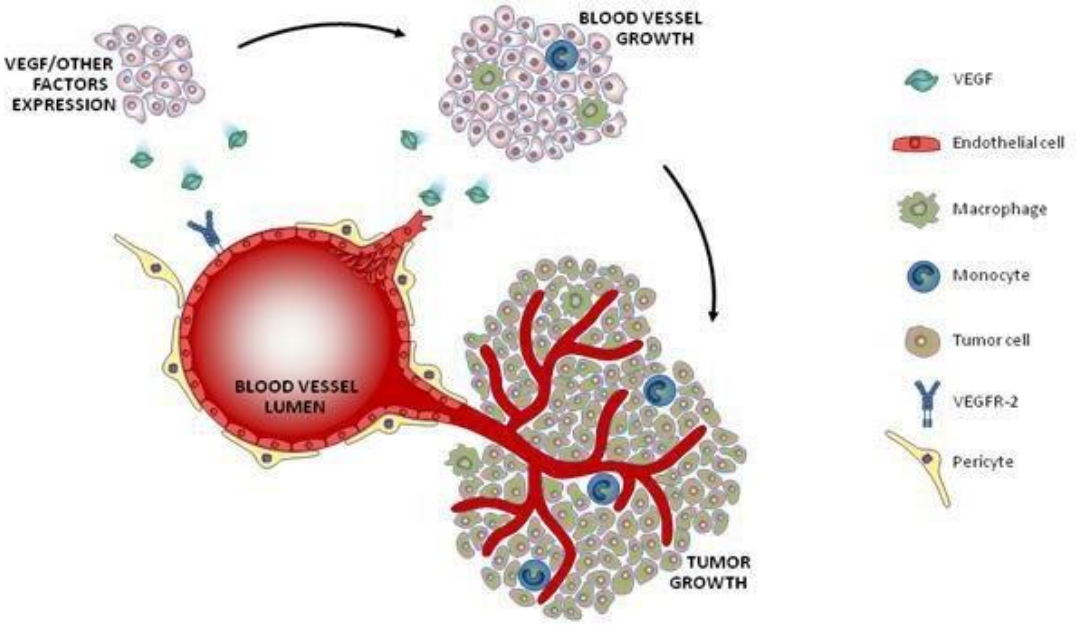
- Most cell types have limited proliferative capacity
  - Capable of ~60-70 division cycles before entering SENESCENCE

- Cellular senescence regulated by TELOMERES
  - Short sequences at the ends of chromosomes
  - Become shorter after each division cycle
    - Once too short, cell enters senescence
  - Telomeres may be repaired by the enzyme TELOMERASE but not expressed by most normal cells

- Production of telomerase means neoplastic cell can continue to proliferate indefinitely

5. Sustained angiogenesis

- Neoplasms requires additional blood supply to grow beyond 1-2mm in size
  - Induce neoangiogenesis or vasculogenesis via:
    - ✓ Persistent hypoxia
    - ✓ Promoting secretion of angiogenic factors (eg. VEGF)
    - ✓ Suppressing angiogenesis inhibitors (eg. thrombospondin-1)
  - Fast-growing tumours may still outgrow blood supply, leading to necrosis



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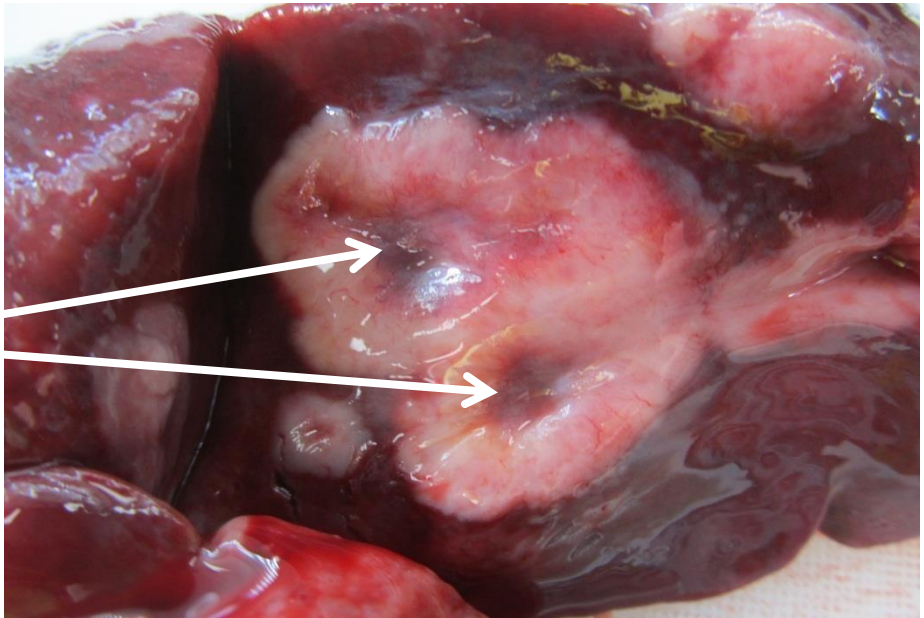
Central necrosis due to poor blood supply



6. Altered cellular metabolism

- Normal tissues:
  - Produce ATP via oxidative phosphorylation under aerobic conditions (uses oxygen)
  - Produce ATP via glycolysis under anaerobic conditions (uses glucose)
- Neoplastic tissues:
  - Produce ATP via glycolysis under both aerobic AND anaerobic conditions

Aerobic glycolysis is inefficient compared to oxidative phosphorylation

BUT…

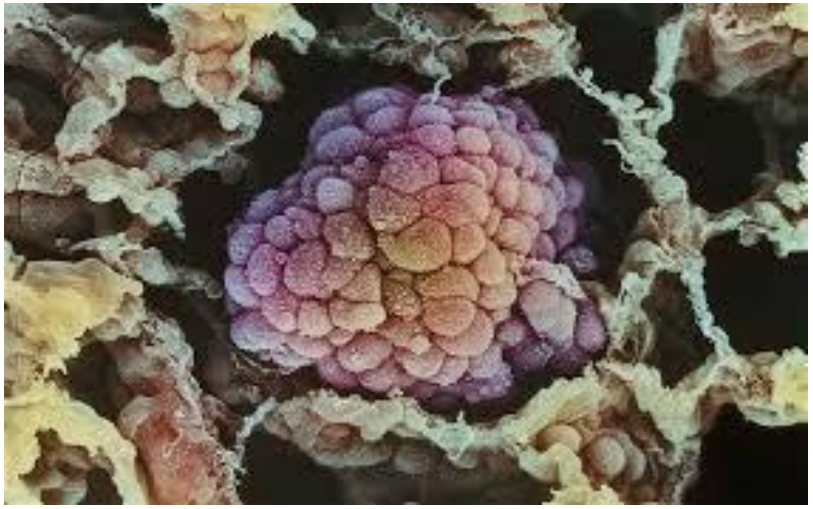
it allows neoplastic cells to proliferate regardless of oxygen levels

(tumours often have intermittent hypoxia, but glucose always available) and

produces substrate chemicals for cell growth (eg. acetyl-CoA, NADPH)

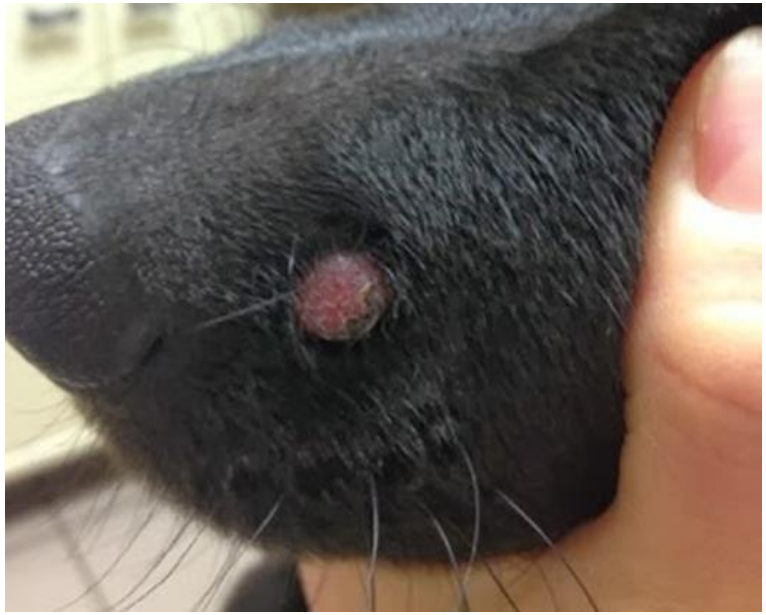
7. Evasion of immune system

- Cytotoxic T cells can recognise and eliminate neoplastic cells
  - Recognition of abnormal or aberrantly expressed antigens on tumour cells
- Successful neoplasms avoid this:
  - Selective survival of cells that do not express immunogenic antigens
  - Suppress antigen presentation to immune system (eg. reduced surface expression)
  - Secrete immunosuppressive factors (eg. TGF-β)

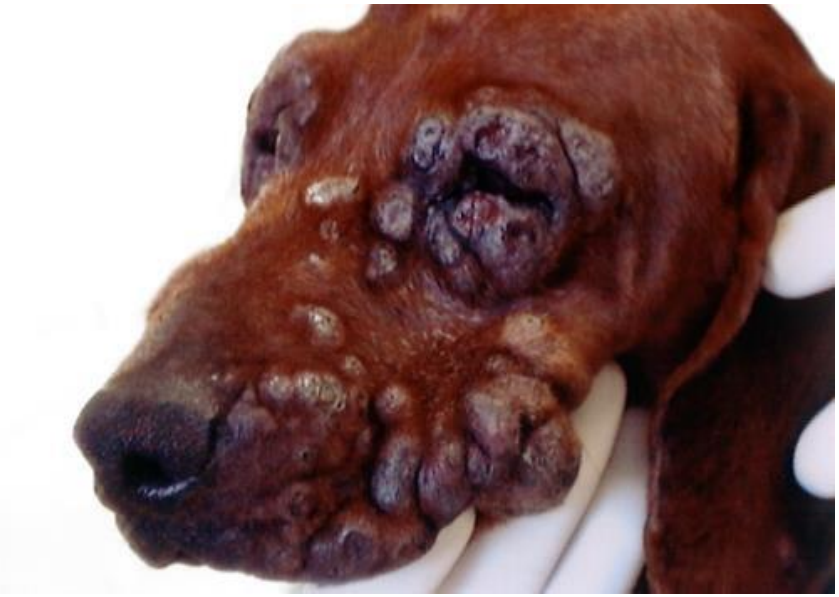


7. Evasion of immune system

- Histiocytomas
  - ✓ Histiocytic neoplasm detected and destroyed by immune system
- Langerhans cell histiocytosis
  - ✓ Histiocytic neoplasm not detected by the immune system, proliferates and metastasises



Histiocytoma



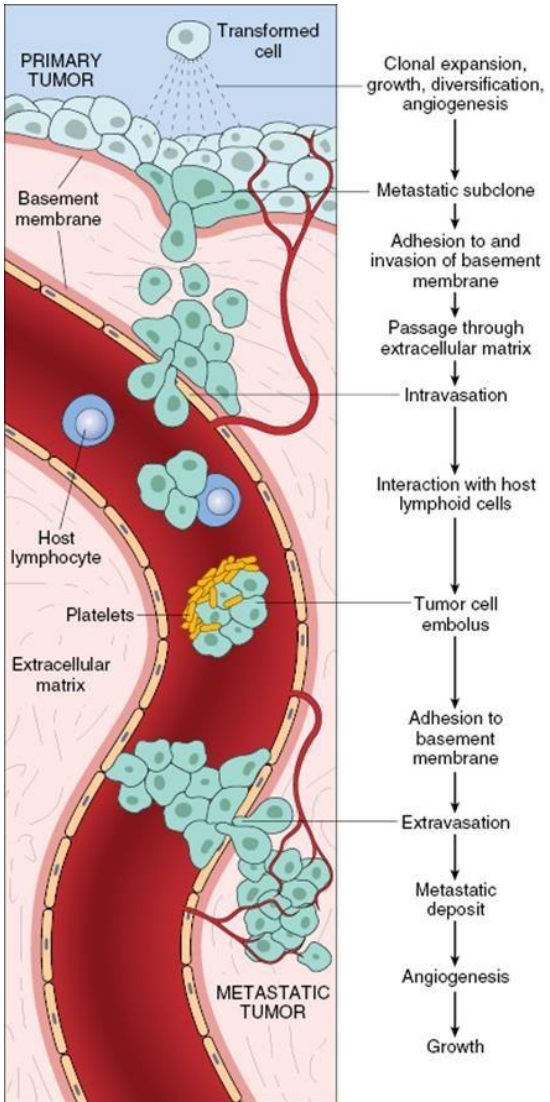
Langerhans cell histiocytosis

7. Evasion of immune system

- Contagious neoplasms
  - ✓ Devil facial tumours
  - ✓ Transmissible venereal tumours
- Animals do not recognise the neoplastic cells as foreign

8. Tissue invasion and metastasis

- Multiple adaptations required
  - Invasion
    - ✓ Loosening of cell-cell contact
    - ✓ Expression of enzymes (eg. matrix metalloproteinases) to break down ECM
    - ✓ Cell motility and migration
  - Metastasis
    - ✓ Entry and survival in blood/lymphatics
    - ✓ Expression of adhesion factors at metastasis site
    - ✓ Survival and growth at new site



Vet Pathol **40**:382–394 (2003)

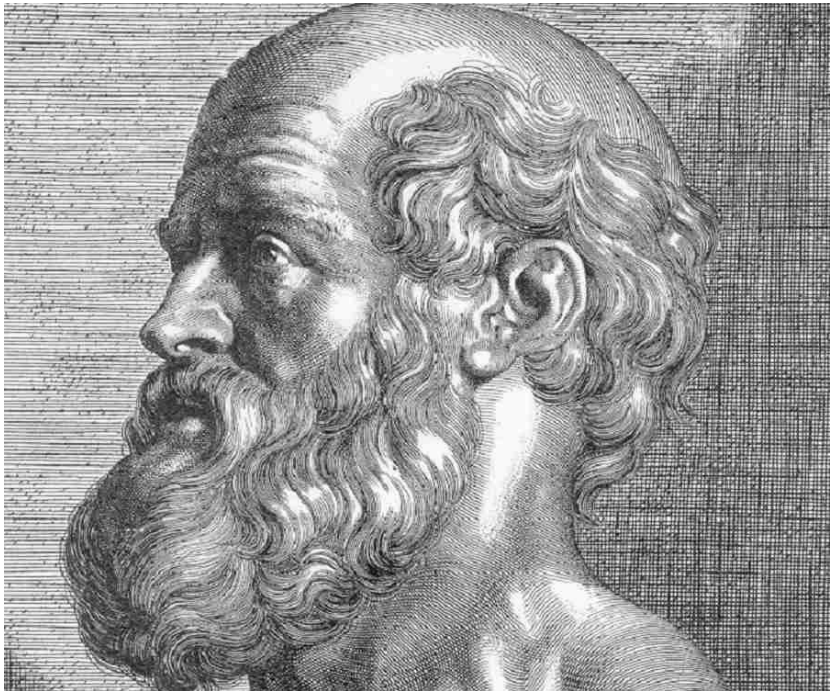
Matrix Metalloproteinase-2 and -9 Involvement in Canine Tumors

P. Loukopoulos, B. A. Mungall, R. C. Straw, J. R. Thornton, and W. F. Robinson

School of Veterinary Science, The University of Queensland, Brisbane, Queensland 4072, Australia

Causes of neoplasia

- Carcinogens
  - Agents that can cause neoplasia
    - ✓ Chemical
    - ✓ Radiation
    - ✓ Microbial
- Inherited
- Spontaneous



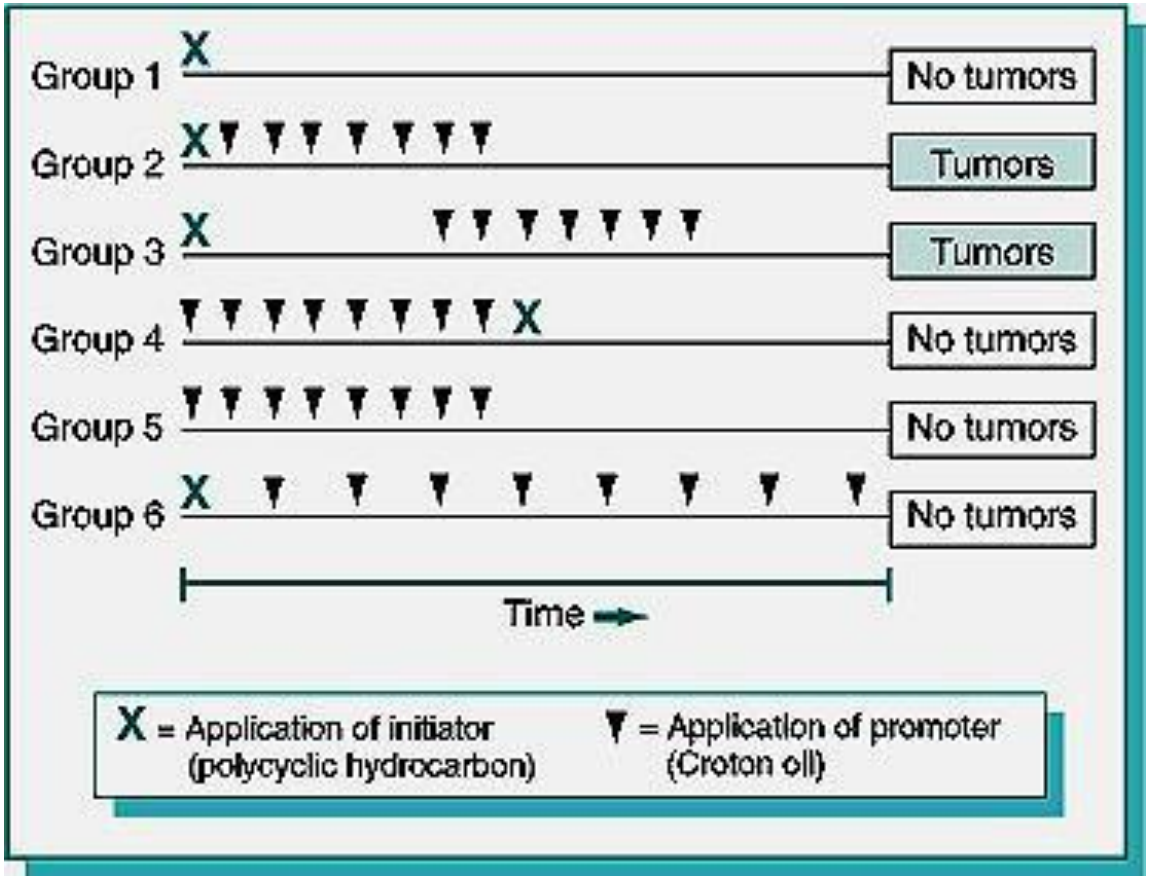
Hippocrates believed that neoplasia was caused by an excess of “black bile”. This belief persisted for 1300 years!

Chemical carcinogens

- Categories of chemical carcinogens
  - Initiaters (eg. nitrosamines)
    - ✓ Directly cause DNA mutation (mutagens)
    - ✓ Usually electrophilic substances causing DNA cross-linking
  - Promoters (eg. oestrogen)
    - ✓ Promote survival and proliferation of mutated cells
    - ✓ Allows accumulation of additional mutations in susceptible population
    - ✓ Examples include hormones or irritants causing hyperplasia
  - Complete (eg. cantharidin, methylcholanthrene)
    - ✓ Have both initiating and promoting attributes

Chemical carcinogens

Carcinogen interactions



Chemical carcinogens

- "Soot wart"
  - Squamous cell carcinoma of the scrotum
  - Seen in chimney sweeps due to soot exposure

Chemical carcinogens

- Glyphosate
  - Recently classified as probable carcinogen
  - Safety trials run by Monsanto found to be seriously flawed or outright fraudulent
  - Courts have awarded total of US$2.4 billion to four Roundup users who developed lymphoma
    - 13,000 cases pending

Veterinary chemical carcinogens

Tobacco smoke

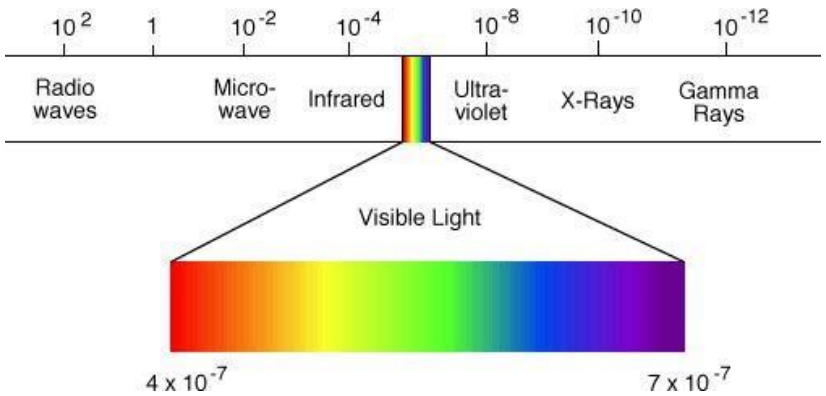
Aflatoxin B (mouldy corn and peanuts)
→ hepatic neoplasia

Asbestos →mesothelioma

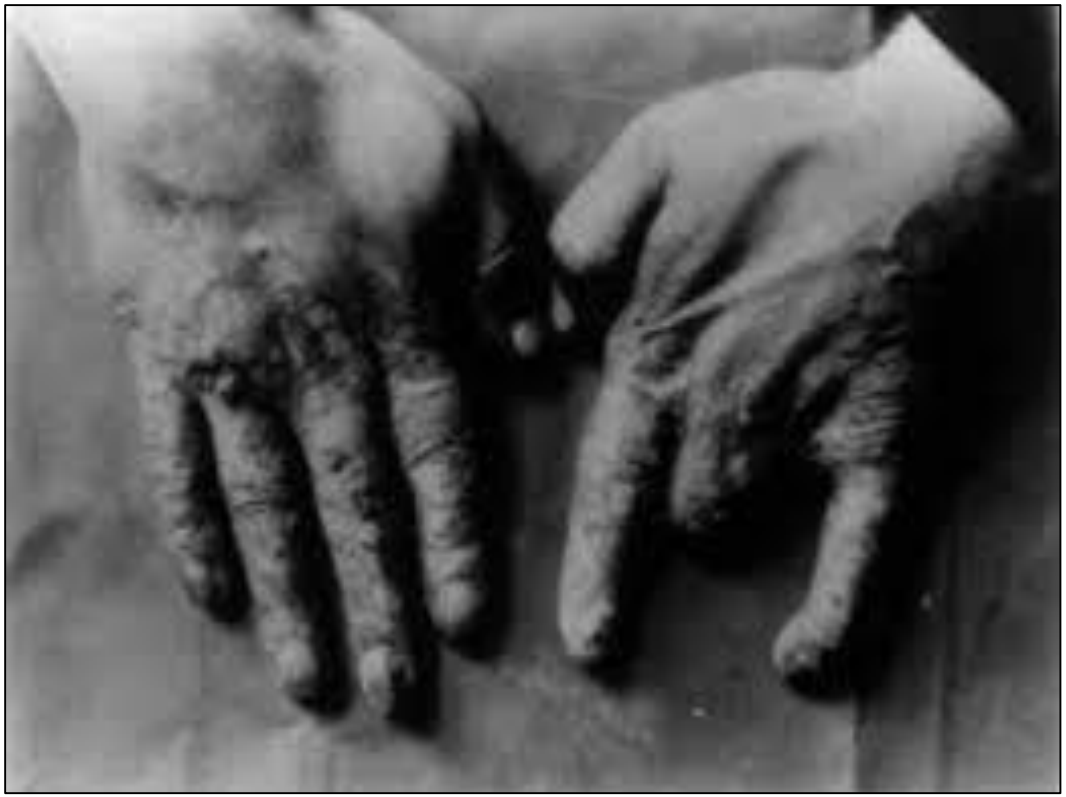
Bracken fern → urinary bladder carcinoma

Radiation

- Typically needs sufficient energy to ionise tissues (break chemical bonds in DNA)
  - ✘ Radio waves (incl. 5G!), microwaves, infrared
  - ✓ X-rays, gamma radiation
  - ✓ Particle radiation (protons, neutrons, alpha particles)
- Ionisation causes:
  - Chromosome breakage
  - Chromosome rearrangement (translocations, inversions, deletions etc.)



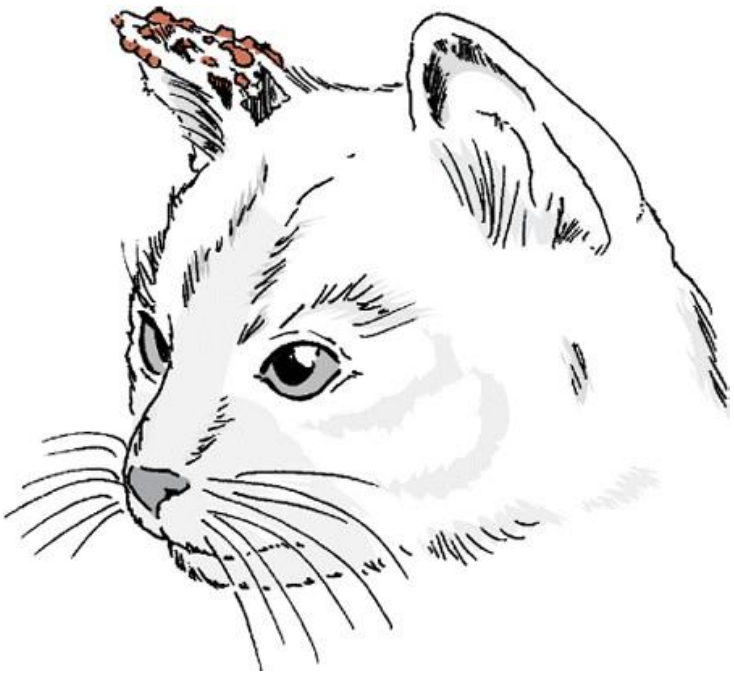
Radiation – X-rays



Dermatitis and cancer on the hands of Clarence Madison Dally, assistant to Thomas Edison during his research on X-rays

Radiation - UV

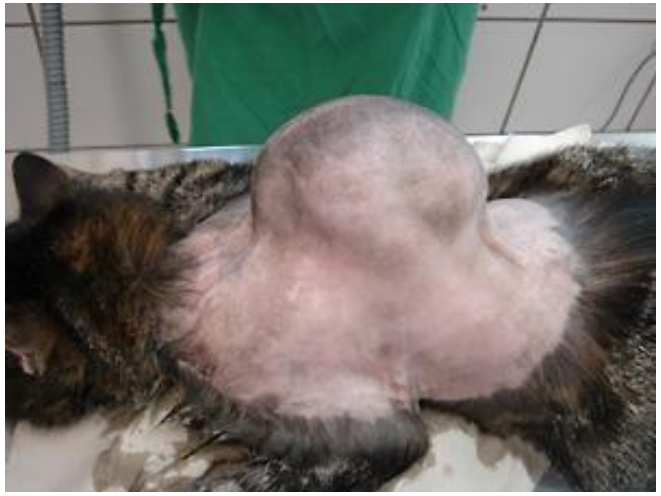
- Not technically ionising but damages DNA through free radical production
- Melanin dissipates UV-B rays
- White cats susceptible to development of squamous cell carcinoma on unhaired areas of the body (ears, nose)

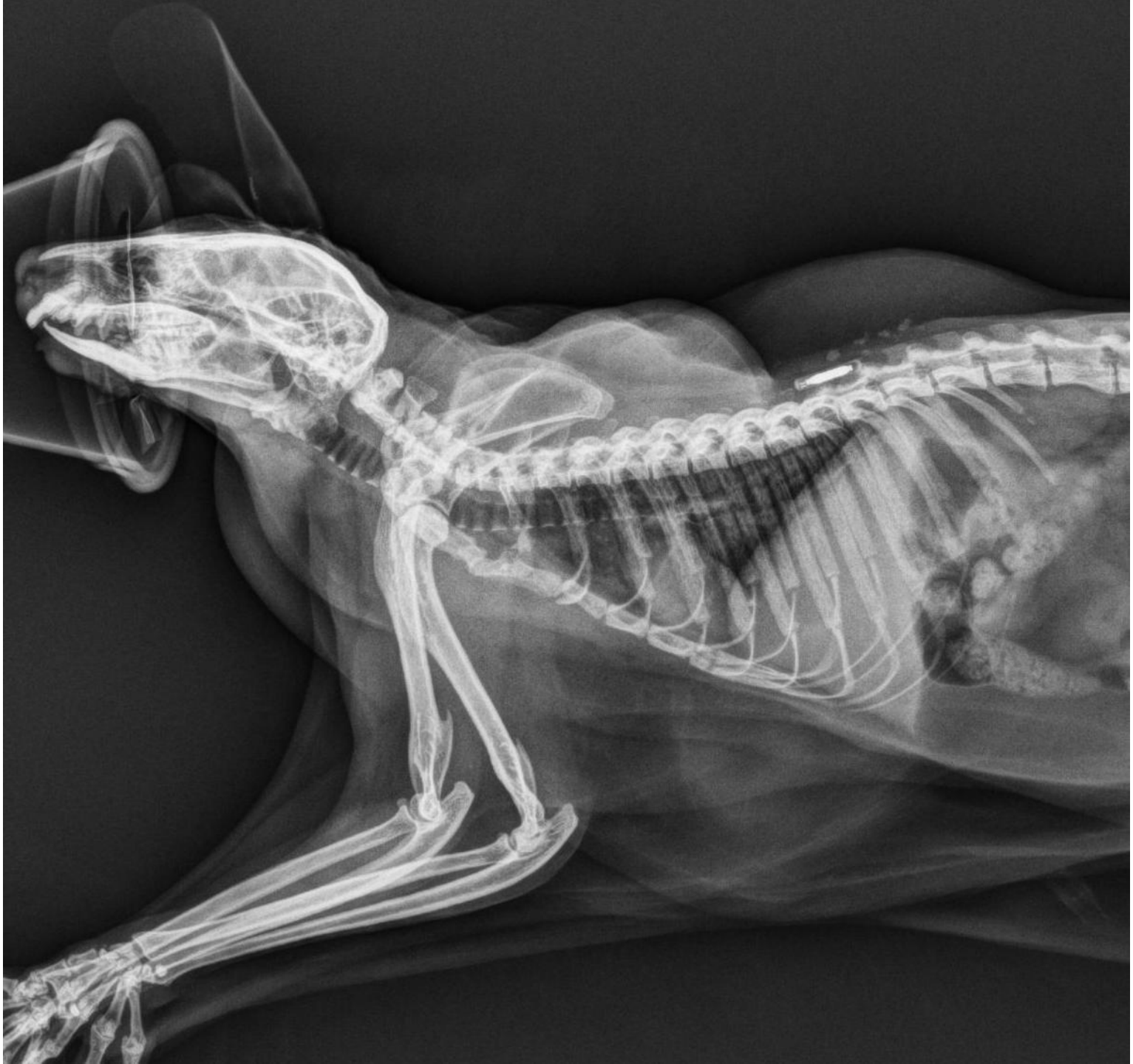


Microbiological carcinogens

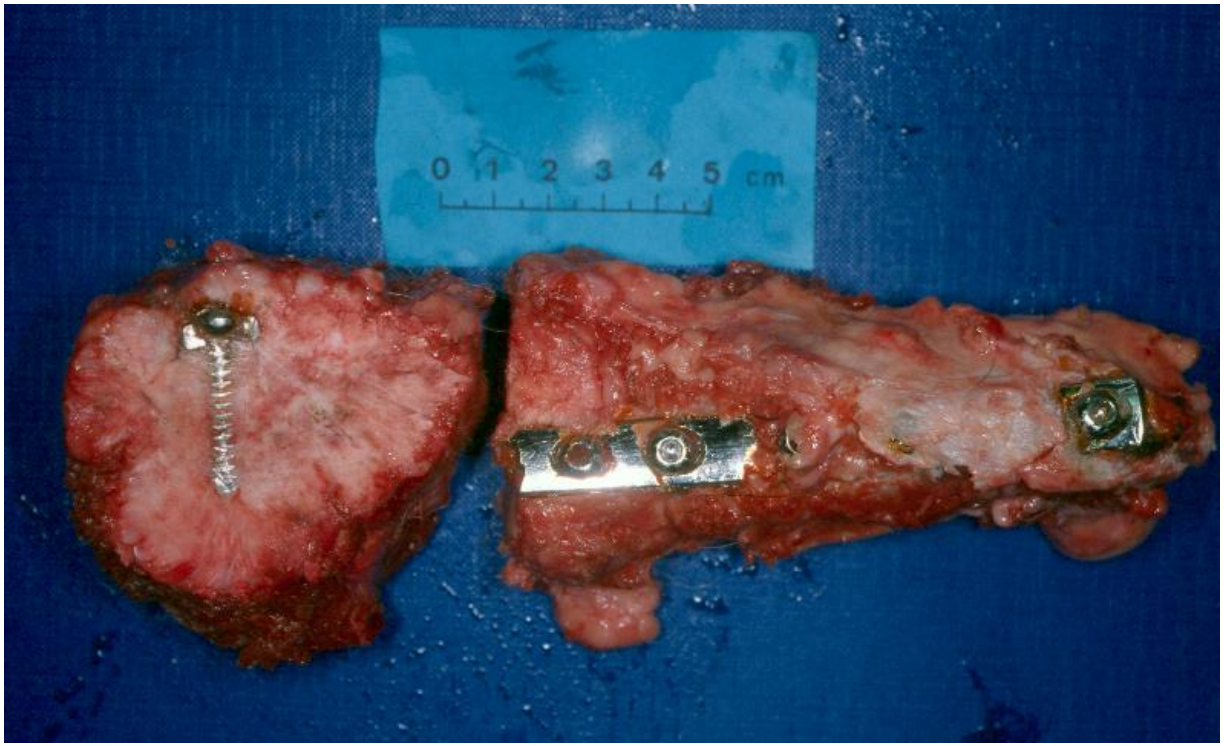
- Direct
  - -Promotion of host oncogene expression
    - ✓ Retroviruses (eg. feline leukaemia virus)
  - -Inhibition of host tumour suppressors
    - ✓ Papillomaviruses

      Accelerate degradation of p53 and Rb
  - -Viral genome contains oncogene that mimics host transcription factors
    - ✓ Herpesviruses (eg. gallid herpesvirus 2)
      - ✓ Marek's disease in chickens
- Indirect
  - Chronic inflammation causes oxidative DNA damage
    - ✓ Hepatitis viruses
    - ✓ Vaccination – feline vaccine-associated sarcoma
    - ✓ Microchip implantation eg in gliders





Osteosarcoma associated with orthopaedic devices



P.Loukopoulos

That's it!

- Now for exams…