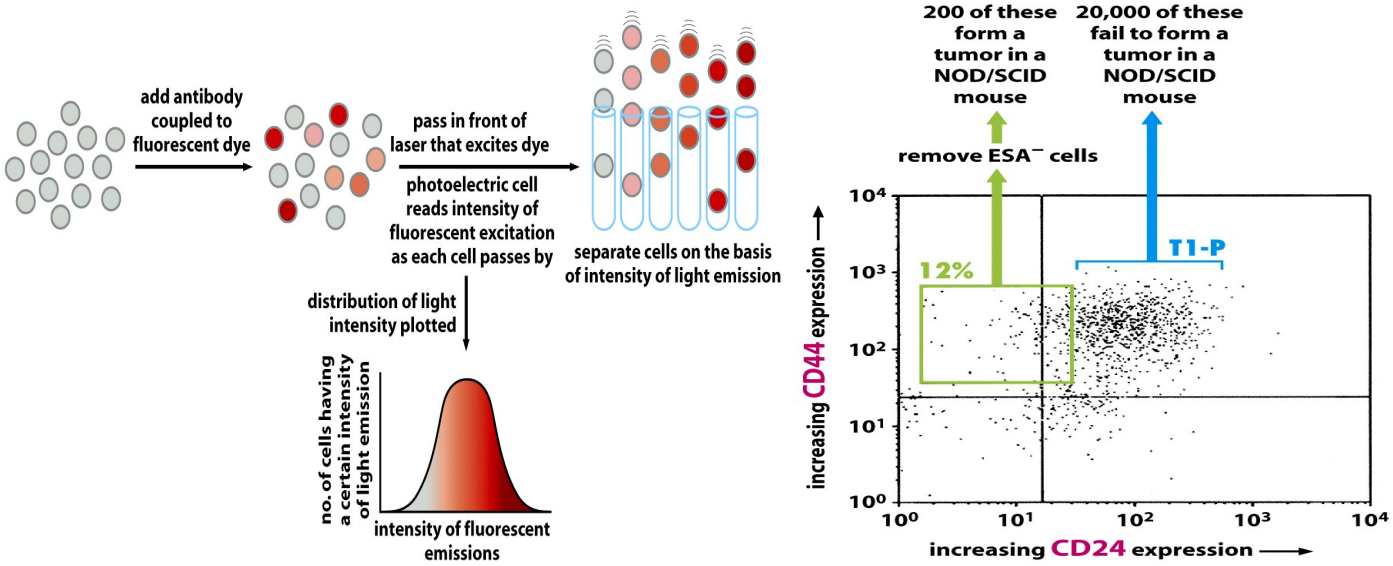
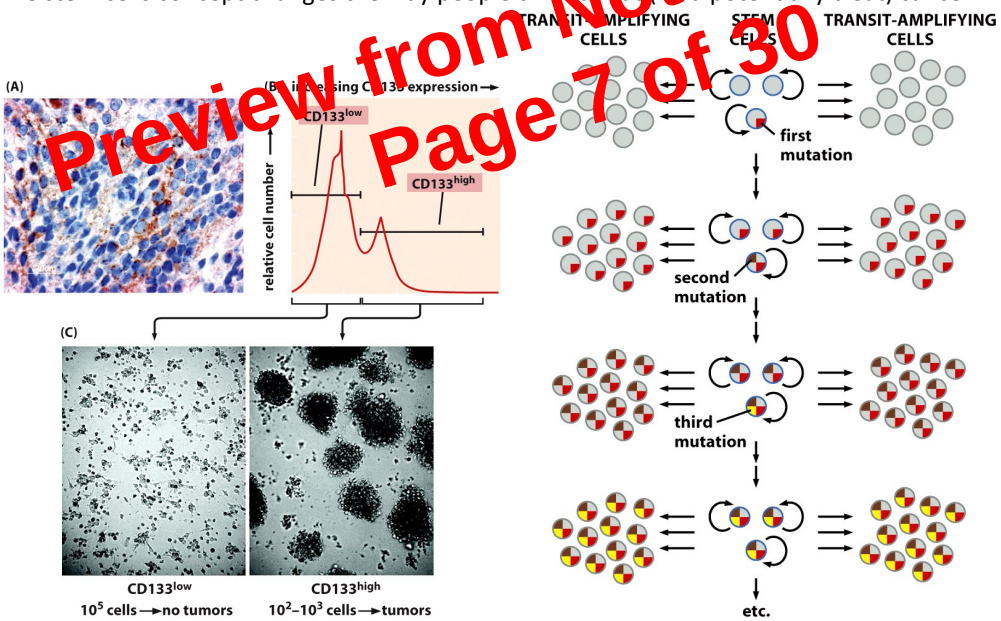
- a small foci of potential breast cancer stem cells stained with an antibody to a cell surface marker the detects cancer stem cells (top right)

The existence of cancer stem cells is somewhat controversial

- The concept is based party on experiments using membrane protein markers to sort out cancer cells into different populations – some population are more tumorigenic in nude mice than others



- breast cancer cells are sorted for CD44 and CD24 expression (membrane protein "markers")
  - cells with high CD44 and low CD22 expression are highly tumorigenic, whereas the majority of breast cancer cells do not form tumors
  - the CD44 high/CD24 low cells maybe enriched for cancer stem cells
- CD133 positive cells may be brain cancer stem cells
- The stem cells concept changes the way people think about (and potentially treat) cancer



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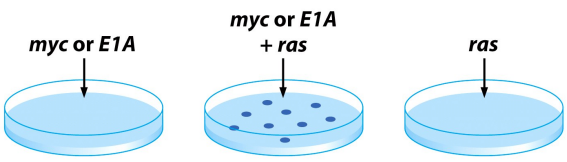
Oncogene Cooperation

Certain sets of oncogenes can work together to transform a normal cell

- These experiments were first done with low-passage rat embryonic fibroblasts or baby hamster kidney cells (NOT NIH 3T3 cells)



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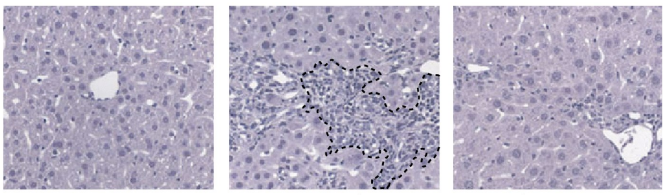
- the use of low-passage primary rodent cell lines revealed cooperating oncogenes

- Nonsteroidal anti-inflammatory drugs like aspirin decrease cancer risk at multiple tissues
- One aspirin a day
  - Lung cancer risk: 0.68
  - Breast cancer risk: 0.7
  - Colorectal cancer: 0.35 (young men)

- Mdr null mice
  - Develop liver inflammation that ultimately leads to cancer
  - Inflammation and cancer development can be suppressed by the NSAID ibuprofen



wild type ***mdr*** $^{-/-}$ ***mdr*** $^{-/-}$ **+ ibuprofen**

  - Inflammatory cells secret cytokines and other inflammatory mediators that promote the survival and proliferation of transformed cells

NSAIDS

NSAIDS decrease the odds of getting esophageal cancer by almost 50%

Common nonsteroidal anti-inflammatory drugs (NSAIDs)

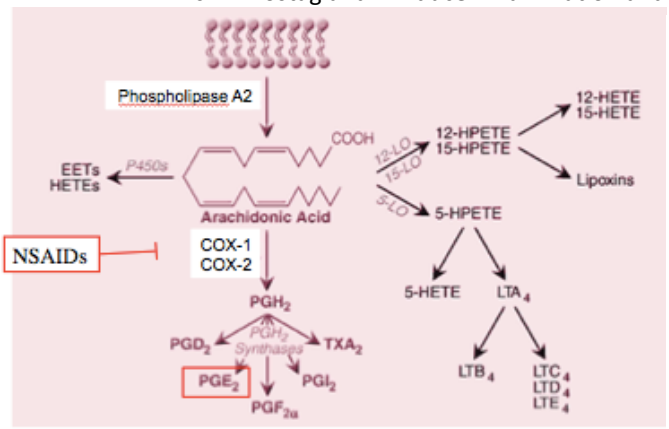
Generic Name	Brand Names
Aspirin	Made by several companies
Ibuprofen	Motrin®, Advil®, Motrin IB®
Naproxen	Naprosyn®, Aleve®
Nabumetone	Relafen®

Source: American Academy of Family Physicians, 2008

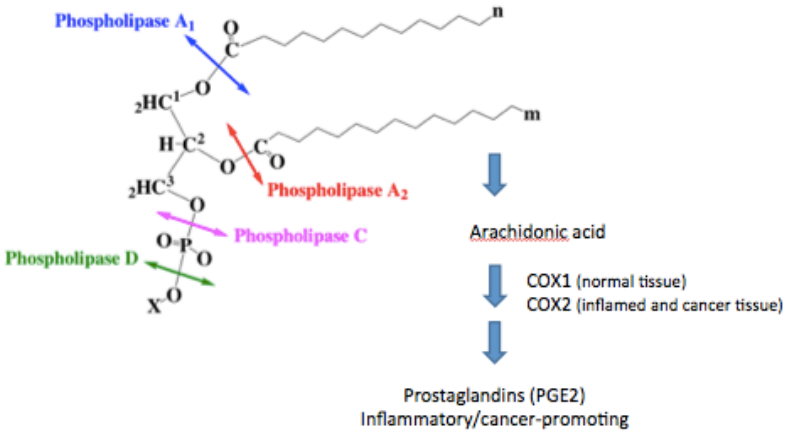
- Acetaminophen/Tylenol is not an NSAID – it is analgesic but not anti-inflammatory, Steroidal anti-inflammatory drugs do not prevent cancer

28) What enzyme(s) do NSAIDs and coxibs inhibit? What product of this enzyme is particularly relevant to cancer promotion?

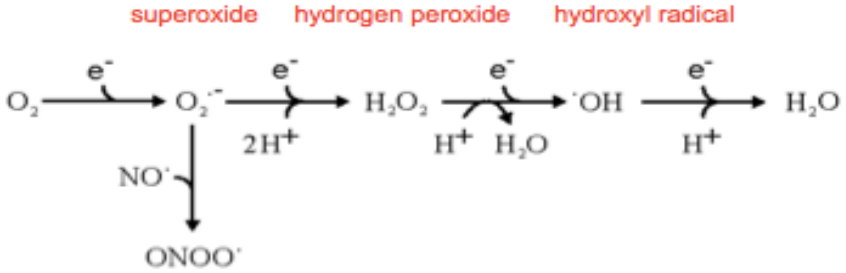
- NSAIDs suppress inflammation and cancer by inhibiting cyclooxygenase (COX) activity and prostaglandin production
  - Prostaglandin induce inflammation and promote proliferation and survival of transformed cells



Tumor-promoting



43) What products are formed from single electron reduction of O2 to H2O?

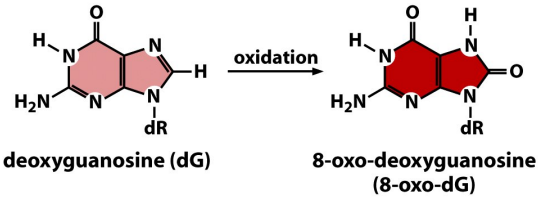


44) Name 3 sources of reactive oxygen species in living tissue.

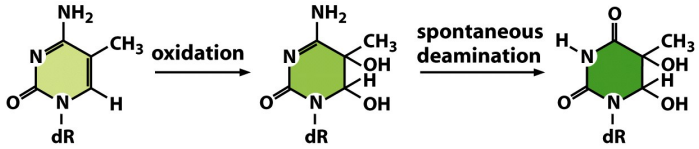
Reactive oxygen species are generated by

- Mitochondria
- Peroxisomes
- Macrophages and neutrophils during an inflammatory response

ROS can damage DNA in many ways, including strand breakage and base oxidation



deoxyguanosine (dG) — 8-oxo-deoxyguanosine (8-oxo-dG)



deoxy 5-methyl-cytosine (d 5'mC) — deoxythymidine glycol (dTg)

45) Would you expect a mouse with a deleted 8-oxo-deoxyguanosine glycosylase to have a higher or lower cancer risk?

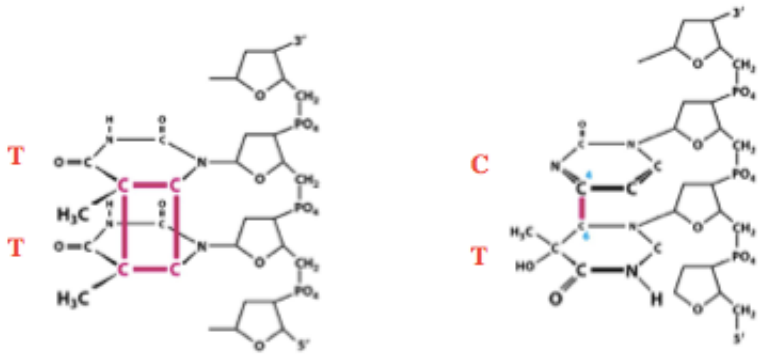
- Oxidation of guanosine can lead to the formation of 8-oxo-guanosine
- If un-repaired, 8-oxo-guanosine can be read as a "T" by DNA polymerase leading to a G to A transition (C to T to C would be a transversion)
- 8-oxo-guanosine is a potentially carcinogenic lesion
  - If you prevent its repair in mice, **their risk of cancer will increase**
- Loss of expression of enzymes involved in repairing 8-oxodeoxyguanosine reduces breast cancer survival (evidence that 8-oxo-dG accelerates progression)

46) Compare how X rays and UV damage DNA

- Chemical and physical damage of DNA by environmental agents is responsible for most cancers
  - X-radiation is ionizing radiation.
  - X-ray can hit water and make ROS.
  - A direct hit to DNA will cause strand breakage
  - UV radiation is a more common source of DNA damage, particularly to skin cells

47) What are the two major photoproducts formed by UV radiation?

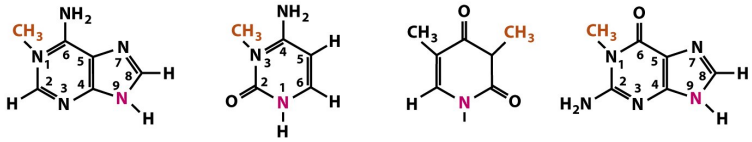
- Common DNA photoproducts include: cyclobutane dimers and 6-4 photoproducts between adjacent pyrimidine's



48) Many environmental agents can alkylate DNA. What is the specialized repair mechanism used by the cell to repair this type of damage?

DNA alkylating agents

- Are electrophiles that modify bases and cause them to be misread by the DNA polymerase
- A number of carcinogens are DNA alkylating agents
  - One example is mustard gas



1-methyladenine **3-methylcytosine** **3-methylthymine** **1-methylguanine**

Genomic instability

- Mistakes during replication and spontaneous base changes (due to chemical instability of bases) can contribute to genomic instability
- Environmental agents make and even larger contribution to genomic changes that lead to cancer

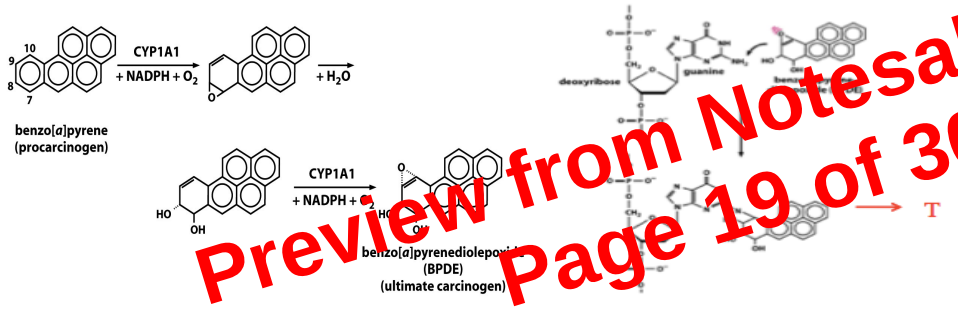
48) Many carcinogens enter the body as non-reactive pro-carcinogens. How do they become carcinogens?

Pro-carcinogens

- Most carcinogens enter the body as non-relative molecules known as pro-carcinogens

49) Why do cytochrome P450 enzymes attach oxygen molecules to pro-carcinogens?

- Cytochrome P450 performs this catalysis. To eliminate these agents, cells first make them water soluble by adding electronegative oxygen to them
- However, addition of oxygen will make some molecules highly reactive carcinogens that will attach to DNA



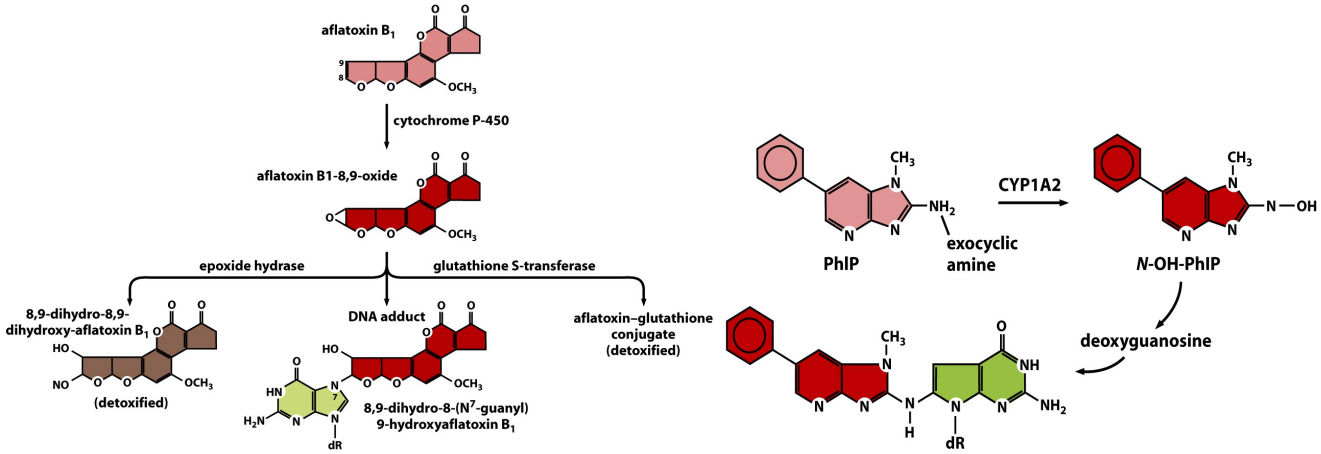
51) List three cytochrome P-450 substrates that are thought to be human pro-carcinogens and indicate where they come from.

1) Benzoapyrene (G→ T transversion)

- Benzo [a] pyrene from **tobacco smoke** is metabolized to **BPDE in the lung**
- BPDE binds to guanine, causes it to be read as a "T during DN replicatioin
- This lead to a G to T transverison

2) Aflatoxin B

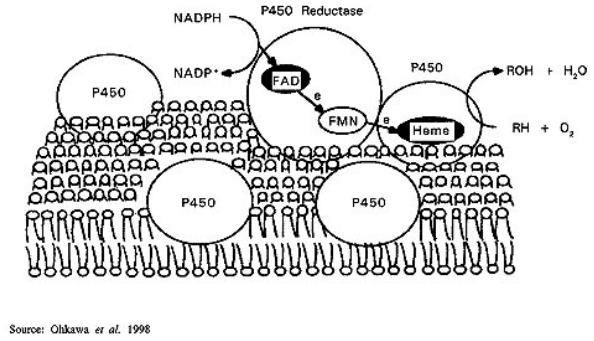
- **Is formed by mold**s that grow on improperly stored grains
- It is activated in the **liver**, where detoxifying pathways compete with DNA reactivity
- DNA reactivity consistent with the AGG to AGT mutations found in the p53 gene in individuals exposed to alfatoxin B
- Cytochrome P-450s performed the phase I metabolism. Phase II detoxification enzymes follow.

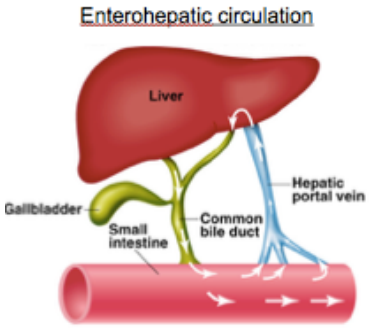


3) Heterocyclic amines (HCAs)

- Like PhIP are derived from **broiled and charred meats**
- PhIP and other HCAs may contribute to the elevated risk of western cancers, such as prostate cancer

Cytochrome P450 enzymes are present on the smooth ER



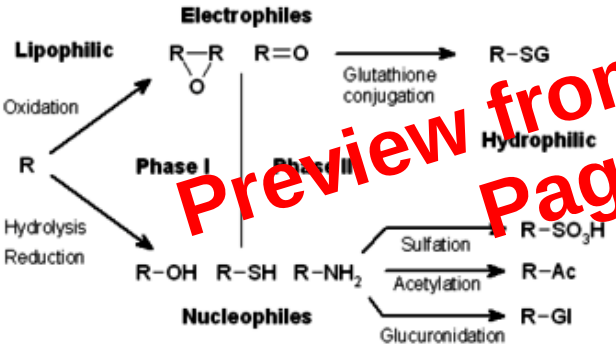


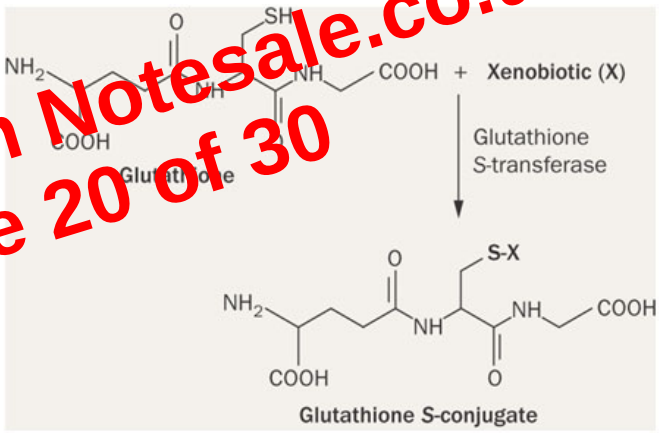
52) Many dietary pro-carcinogens are absorbed by the small intestine. What it their next destination and where does most of their metabolism occur?

- Most cytochrome P450 and phase II enzyme metabolism occurs in the liver
- Blood from the small intestine flows through the liver, which serves as a filter of sorts
- Some of the pro-carcinogens maybe missed by the liver, or the metabolized compounds may find their way to a distal organ

53) What are Phase and I II enzymes?

- Cytochrome P450 enzymes are phase I enzymes
- Phase II enzymes conjugate metabolites to small molecules for secretion in the urine or bile





54) What is glutathione and how do glutathione-S-transferases protect cells from carcinogens?

Glutathione S-transferase

- Phase II detoxifying enzyme
- It links glutathione to the Xenobiotic/carcinogen, which facilitates its excretion in the urine or bile (top right)
- Glutathione conjugation is one of a number of phase II reactions involved in carcinogen detoxification

Mechanism	Involved enzyme	Co-factor	Location
Methylation	Methyltransferase	S-adenosyl-L-methionine	Liver, kidney, lung, CNS
Sulphation	Sulfotransferases	3'-phosphoadenosine-5'-phosphosulfate	Liver, kidney, intestine
Acetylation	N-acetyltransferases Bile acid-CoA:amino acid N-acyltransferases	Acetyl coenzyme A	Liver, lung, spleen, gastric mucosa, RBCs, lymphocytes
Glucuronidation	UDP-glucuronosyltransferases	UDP-glucuronic acid	Liver, kidney, intestine, lung, skin, prostate, brain
Glutathione conjugation	Glutathione S-transferases	Glutathione	Liver, kidney
Glycine conjugation	Acetyl Co-enzyme As	Glycine	Liver, kidney