

IMMG Review:

Lectures 1-3 Chambers

Blood clotting – a hole is closed by platelets and fibrin, and scab forms to keep bacteria and dirt out, while WBC's clean it – takes one hour. Skin cells migrate to trauma site to repair cut.

CAD – when macrophages surround cholesterol accumulations and restrict blood flow. Statins stop **HMGCoA reductase**, reducing cholesterol (HDL should be >40, and LDL <100), and preventing CADs. They are also anti-inflammatory and decrease bone loss (good for osteoporosis) and stabilize plaques so they don't break off, clog off an artery, and cause something like a stroke, arrhythmia, or MI (myocardial infarction). Statins also help Alzheimer's patients because the beta-amyloid plaques have cholesterol in them. To get rid of these clots, we use **TPA** (tissue plasminogen activator) that dissolves clot (via **plasmin**) and **balloon angioplasty w/stent** (that breaks off clot and holds artery open).

Platelets - anucleate, so have all the proteins and GFs already w/in them. If platelet count really low (<70,000/mm³ – normal is 300-500K) then patient is hemophiliac. Shape is of a disc that becomes circular when activated. Its activated by **ADP, ATP, serotonin, and Ca²⁺, Epi, and NE**. It has **integrins, fibrinogen, fibronectins, and Von Willebrans factor (vWF)**.

Clotting – trauma to epithelium causes collagen to be exposed and ADP released. ADP along w/Ca causes aggregation. **GP2P3a (an integrin)** – binds fibrinogen, which is converted to fibrin (main event in clotting) which is used for platelet-platelet interaction. Clot retracts to fit size of wound.

vWF mediates adhesion of platelet to site of trauma via collagen. This happens *before* platelet-platelet adhesion, and involves vWF binding with **GP1b**. vWFs are usually floating in blood bound to **factor VIII**, but endothelial cell injury causes their release from endothelial cells as well. All this aids in clotting.

Platelets also release **PDGFs** (Platelet derived growth factors) and **chemotactic** factors (to recruit leukocytes).

When platelets are activated, they release a bunch of stuff mentioned before, as well as **factor 13**, which stabilizes fibrin. Platelets clog holes and provide phospholipid surface for clotting to occur. ADP released during epithelial cell damage causes **integrins (GP2P3a)** to be formed, and **Thromboxane A2** (a prostaglandin formed via the action of a cyclooxygenase [COX]) to be formed. Therapies to prevent blood clotting target these two things. **Thrombin** makes **fibrinogen** to **fibrin**, forming the clot.

The primary homeostatic plug is the collagen that is released when there is injury. The platelet-fibrin plug is the secondary homeostatic plug.

Also, anything that causes cAMP levels to rise inhibits platelet aggregation.

So, therapies are aimed at decreasing Thromboxane A2 and other prostaglandins via inhibiting these COX enzymes. Aspirin inhibits COX1 and COX2, but our stomach has COX2, so aspirin causes problems there. So, researchers wanted to use only COX2 inhibitors because they target arthritis immediately w/o causing stomach problems. However, endothelial cells have COX2 and made a prostaglandin called prostacyclin, which increases cAMP levels, and as discussed above, this would inhibit platelet aggregation. Well, that's why all the problems happened w/Vioxx and Celebrex.

Tylenol is given instead of aspirin after surgery because it's a COX3 inhibitor, and so, doesn't have the bleeding problems that are associated w/aspirin (COX1 and 2 inhibitor)

There are a lot of ways that endothelial cells stop platelet aggregation so that we don't get clogged arteries: **NO**, which causes vasodilations, **PG12 prostacyclin**, which causes an increase in cAMP (as mentioned above), and **ADPases** (because ADP activates Thromboxane A2 and integrins (GP3P2a)). It makes **thrombomodulin** which via **protein C and S** destroy factors **Va and VIIIa**. It makes **anti-thrombin via heparin**, and activates plasmin (which destroys fibrin and dissolves clots).

Clots are a three-component system:

- Generation of thrombin
- Conversion of fibrinogen to fibrin
- Stabilization of fibrin

The systems that do this are **extrinsic**, which is tissue derived (and is the one aiding in pro-thrombin turning into thrombin), and **intrinsic**, which has to do with molecules floating around.

Extrinsic: factor VII is converted to factor VIIa by tissue factors (calcium needed), which converts X to Xa, and Xa along with Va activates prothrombin. These clotting factors need to be glued to the injury site. This glue is provided by **gamma-carboxyglutamic**

acid (the 'glue' made from glutamic acid via a carboxylase – **this carboxylase is activated by vitamin K**). **Warfarin** antagonizes vitamin K and is thus a rat poison (they bleed to death). **Coumadin** is a derivative of Warfarin.

Factors V and VIII work **both in the intrinsic and extrinsic** systems. They use the 'glue' as a scaffold for the reactions to take place. w/o them, **there is no reaction**. We have already seen how Va works w/Xa to activate prothrombin into thrombin. Thrombin does not use the 'glue' because if it did, it would be constitutively available and turned on, thus never stopping the clot. So, when thrombin starts working, the 'glue' is removed. **So, prothrombin has the 'glue', and thrombin does not.**

Intrinsic: starts w/allosteric activation **factor XII**. XII activates XI, which activates IX, which together with VIII converts X to Xa. This is where the intrinsic and extrinsic systems converge. The extrinsic system is the main one in clotting, and the intrinsic one is helpful but *not* necessary. The reason is that VIIa can activate IX, which can activate X with the help of VIII. So, XII and XI are NOT necessary. Also, XII is involved in *plasmin* activation, which *dissolves clots!!* So, its necessary to maintain a clot free environment – w/o it people get heart attacks.

You need Ca^{2+} as mentioned before to clot, so you can add a calcium chelating agent like citric acid or EDTA to stop blood from forming clots.

Last point – I mentioned earlier that factor 13 is used for clotting – it aids in fibrin cross-linking, and it's a **transglutaminase reaction**. All other reactions mentioned are **serine protease** reactions – so, they can be inhibited by **serine protease inhibitors**.

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Lecture 25 Mechanisms of Mutagenesis Kaufman

Mutation: random change in genetic material, all variation springs from this, can affect somatic or germline cells.

3 Types of Mutation:

1. **Genome Mutation:** (MOST COMMON)

Chromosome missegregation can lead to aneuploidy

IE Down syndrome (trisomy 21)

2. **Chromosome Mutation:** (2nd Most Common)

Chromosomes exchange pieces of DNA

Homologous portions at inappropriate times and recomb occurs

Translocation: exchange of piece of DNA for another. If same length: "balanced"

3. **Gene Mutation:** (Least Common)

Caused by base mutations. Infrequent bc of repair mechanisms

3 Types of Gene Mutations

1. **Point Mutations (Nucleotide Substitutions)**

One base substituted for another

2 types

1. **Missense**

Wrong amino acid transcribed

2. **Nonsense**

Premature stop codon

Types of point mutations

Transitions:

Purine (ie G) substituted for a purine (ie A) or pyrimidine (ie C) substituted for a pyrimidine (ie T)

Transversions:

Purine (ie G) substituted for a pyrimidine (ie C) or vice versa

2, 3. **Deletions and Insertions**

Can result in **frameshift mutations** if bases added/deleted not in multiples of three.

Frameshift mutation can wipe out the normal stop codon and make huge proteins

Rules of the Genetic Code

1. Codons read from 5' to 3'

2. Codons do not overlap and no gaps

3. Message translated in fixed reading frame set by initiation codon

Consequences of mutation

1. usually loss of protein function

2. sometimes gain of function

3. rarely new function

Mutations and Cancer

1. protein may be expressed ectopically at the wrong place or time

Structure of a Gene

1. not all mutations in exons
 - a. mutations in introns not always silent
 - i. may create a new splice junction changing mRNA and protein
2. Mutations at other places on 5' end
 - a. Enhancer
 - b. Tissue specific elements
 - c. Tata box
 - d. Cap site
 - i. All control the timing, amount, and location of expression

UV Radiation Mutagenesis

1. Mutagenesis requires a biological step
 - UV light causes covalent linkages between adjacent pyrimidines
 - Pyrimidine dimers (T-T, T-C, C-C)
 - 90% are (T-T dimmers)
 - These do not cause mutations bc of A rule
 - A Rule:** Replication continues past lesions
 - Only problem with other 10%

Structure of Hemoglobinopathies

- Two copies of two different polypeptides, alpha and beta (similar to each other)
- They combine to form a tetramer
- The way they interact is very important in function of hemoglobin

Genetic Disorders of Hemoglobin

1. Structural variants
 - a. Hb S (sickle cell hemoglobin)
 - i. Under low oxygen can lead to hemolysis
2. hereditary persistence of fetal hemoglobin
3. thalassemias
 - a. imbalance in production of alpha and beta globins
 - b. MOST common single gene disorder in humans
 - c. Group of diseases in which mut decreases alpha or beta chain
 - i. IE alpha thalassemia
 1. most common forms result from deletions
 - a. this results in misalignment and unequal amount of material exchanged (2 on each chromosome)
 - b. alpha thalassemia genotypes
 - i. normal would have 4 alpha genes
 - ii. normal func could also have 3 alpha genes 75% (considered a silent carrier)
 - iii. alpha thalassemia trait 50%
 1. person inherits one deletion and develops the other
 2. person inherits both deletions (rare)
 - iv. Hb H disease 25%
 1. 1 gene present
 2. severe hemolytic anemia

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Lecture 26 Detection of Mutations Kaufman

cDNA

1. Complementary to mRNA
2. introns have been spliced out.
3. made by isolating mRNA from biological sample using its poly A tail

Hybridization

Two complementary single stranded DNA/RNA forming bonds becoming double-stranded.

Probe

A stretch of single stranded DNA/RNA used to detect complementary strand in sample

Restriction Endonucleases

1. Enzymes that recognize specific double-stranded DNA sequences and cleave the DNA near the recog site.
2. Can detect point mutations in a restriction site (ie sickle cell disease)

Southern Blot

1. A filter to which DNA has been transferred, usually after restriction enzyme digestion and gel electrophoresis. This technique allows for detection of a DNA fragment using a labeled probe.
2. Can detect large molecular defects that are better than just chromosome analysis but not superior enough to reveal single mutations. (ie good for Muscular Dystrophy)

PCR

1. Amplify a given DNA sequence using DNA polymerase and short, synthetic oligonucleotide primers that are complementary in sequence.
2. good for Lesch-Nyhan disease, also Tay-Sachs.

Dideoxy DNA sequencing

DNA sequencing in which template strand is replicated from a particular primer sequence and **terminated** by incorporation of a nucleotide that contains **dideoxyribose** instead of deoxyribose.

DNA sequencing by chain termination method

Chains of different lengths are synthesized in presence of dideoxynucleotides. Length depends on sequence of DNA template. Reading a DNA sequencing gel can give the 5' to 3' sequence.

Northern Blotting

Detect major changes in mRNA levels or structure of a gene but not minor alterations.

ASO (allele-specific oligonucleotides)

1. Technique to find single base-pair mutations. If you want to know if patient has specific mutation.
2. ie good to detect retinitis pigmentosa

PROBABLE EXAM QUESTION may be easier to look on page 10 of the handout or Page 4 2005 coop regarding GEL PATTERNS.

Basically Homozygous BB and AA are going to be one size respectively. AB will have both. The trick is to know what is A (which of the two smaller fragments) in this case, one must look at what the probe is homologous to it.

Polymorphism:

1. variation in population that occurs with a frequency of 1% or more.
2. good to be used as a marker to map things especially at restriction sites.

RFLPs

Restriction fragment length polymorphisms
Not responsible for the disease—neutral change with no functional consequences.
Most useful on individuals who are heterozygous

DNA fingerprinting of twins.

Identical twins would have an identical DNA printing—all others would not

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Lecture 27 Genetic Instabilities Sergei Mirkin

Myotonic Dystrophy

Non Mendelian genetics
Anticipation: increased expression over many generations
Repeats in untranslated part of RNA at 3' end (type 1)

Anticipation

An expanded triplet repeat
Can be tetrameric, pentameric, dodecameric as well
Usually the triplets have no effect
With each generation, number of repeating triplets increased

Fragile X

Anticipation known as Sherman Paradox
CGG on FMR-1 gene
Between 14-200 repeats get fragile X associated syndrome
More than 200 CGG, symptoms of disease state. 3500 most severe.
Repeats in untranslated part of RNA at 5' end

Friedreich's Ataxia

Repeats found in introns

Polyalanine Disease

Caused by CAG expansion
Repeats in exons

ie polydactyly
 Poly-glutamine Disease
 Huntington's disease
 Paternally transmitted
 Hairpin like structures can form
 If DNA is denatured and then re-annealed. Caused by triplet repeats.
 DNA polymerase can add new bases one by one during replication
 If it slips and reads extra triplets, you get an extension.
 If it forgets one, you get a deletion.
 Where does this problem occur?
 Lagging strand is more vulnerable because it is made in fragments
 To prevent hairpin, RPA is used, but has trouble binding to DNA if there is a section of repeats.
 Flap model
 Expansion of triplets during DNA replication. RNA primer has to be removed so
 Okazaki fragments can be ligated. Displace primer using FEN-1. RNA/DNA flap formed.
 If the repeat section is part of the flap, the FEN1 cannot do its job and the flap gets ligated to the next okazaki
 fragment. Causes expansion of the triplet.
 Other causes for initial repeat expansions. 2003 coop
 ORI-switch: replication progresses in opposite direction
 ORI-shift:
 Fork-shift

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Lecture 28 Genetic Instabilities II Sergei Mirkin

Fragile X

Mental retardation, facial dysmorphism
 More severe in males over females
 Maternally transmitted
 Genes are FMR1 and FMR2
 Patients have 230-1000 CGG repeats, 50-230 do not express disease
LOSS of function
 FMRP, RNA binding protein, not expressed
 Transcriptional silencing
 Promoter hypermethylation: more meth, more severe
 Methylation recruits methyl CGG binding proteins
 This attracts histone deacetylase
 This condenses chromatin and gene is turned off
 Why LOSS of function dominant?
 Males only have one X chromosome.
 Females have two but have a 50% the mutated one was deactivated.

Friedreich's Ataxia

Autosomal recessive disease
 Expansion of GAA repeats within an intron of the gene Frataxin
 MOST commonly inherited form of ataxia
 Patients: 112-700 GAA repeats
 Blocks transcription of gene involved in cellular iron metabolism
 Loss leads to damage to mitochondrial DNA and membrane
 Consequently, ataxia

Myotonic Dystrophy

Autosomal dominant
 Expansion of CTG stretch on 3' untranslated part of DMPK gene
LOSS of function
 Symptoms: Cardiac arrhythmia, cataracts, myotonic neuropathy.
 Loss of DMPK gene: cardiac arrhythmia
 Disruption of gene SIX5: cataracts
 Muscle weakness caused by **gain of function** bc mutation causes inactivation of many other genes and splicing.

Huntington's Disease

Autosomal dominant
 Symptoms: involuntary movement, impaired motor control, dementia
 CAG expansion
 Paternal inheritance

Gain of function

Patients with disease: 36-121 CAG repeats

Lectures 29 &30: Inborn Errors of Metabolism

Oculocutaneous Albinism – tyrosinase deficiency

symptoms – lack of pigmentation (hair, skin, retina, iris), nystagmus, decreased visual acuity, lack of stereoscopic vision

confirmatory test – check for tyrosinase activity

explanation – tyrosinase catalyzes tyrosine to DOPA and DOPA to DOPA quinone reactions, which are steps in the synthesis of melanin

inheritance pattern – autosomal recessive (have to get a copy from mom and dad – probability is 25% if parents are heterozygous)

Studying Oculocutaneous Albinism

Cloning Tyrosinase cDNA

1. isolate RNA
2. create cDNA using reverse transcriptase
3. splice cDNA into Lambda phage genome (EcoRI to cleave Galactosidase gene) marker
4. package into phage
5. infect E. coli – E. coli synthesizes protein, some lyse
6. culture transfected E. coli
7. find E. coli culture + for tyrosinase gene using filter paper/Ab
8. cDNA clone isolated

Analyzing Tyrosinase cDNA Clone

1. RNA complementary to cDNA (labelled with ^{32}P) produced in tyrosinase producing cells – melanocytes, but not fibroblasts or lymphocytes

2. also RNA complementary to cDNA (labelled with ^{32}P) produced in wild-type mice, but not in mice with radiation induced tyrosinase deficiency (albinism)

Sequence cDNA

Clone Entire Tyrosinase Gene

1. membrane filter DNA from lambda phage genomic library
2. locate tyrosinase gene via hybridization with radiolabelled tyrosinase cDNA
3. clone

Detecting Oculocutaneous Albinism

DNA sequencing method

amplify exons of involved gene by PCR
sequence exons on gel and look for changes

Allele-specific nucleotide scheme

PCR exon where sequence is located – apply to nylon membrane
create ^{32}P -labelled 19bp oligonucleotides corresponding to wildtype and mutant sequences at key locations
hybridize with exons – 2 separate samples (one wildtype, one mutant)
expose on x-ray film – note film exposure

Treatment of Oculocutaneous Albinism

ameliorate symptoms (that is . . .)

avoid sun

some treatment available for visual impairment

Phenylketonuria

phenylalanine hydroxylase deficiency

screened at birth

treatment is a phenylalanine free diet

major consequence is brain damage/impaired cognitive development

details:

substitution of leu for pro @ codon 81 accounts for 20% of OCA alleles in US whites

cys to arg @ codon 89 may be more common in US blacks

OCA in 1:39,000 Caucasians and 1:28,000 Africans

two of four sites of prolific point mutations responsible for OCA are at copper binding sites, which seems to be essential for enzyme function

terminology:

consanguinity – related by blood (sanguis = blood in latin)

- in terms of genetic inheritance, it means getting two copies of the same

gene (usually a recessive gene – cause that’s what we notice) from the same ancestor, through converging bloodlines

proband – subject of genetic investigation (think, person being probed, not pro-band :)

founder effect - The loss of genetic variation when a new colony is formed by a very small number of individuals from a larger

population – or – as Dr. Kaufman puts it: rare mutations tend to remain within specific populations if most matings occur within that population

genetic heterogeneity- this is the idea that many different types of mutations can cause the same disorder, and many of those will exist in the population

notes:

look at chart on p5 of handout for symbols used to draw pedigrees

Endocrine Diseases (Both lectures)

A lot of this will be repetition from Physio (even if you just read BRS ☺) so I will be quick and brief – I will indicate where the new material starts.

Hormones can be categorized as 1. Peptides which bind to the receptor on plasma membrane and activate intracellular signaling or 2. Steroid Hormones that like to go inside into the cytoplasm and bind to DNA binding domains and act like a Transcription Factor.

Pituitary gland- Anterior Pituitary comes from glandular epithelial cells – Rathke’s Pouch while Posterior Pituitary is from neuroectoderm.

Where is it located? Sella turcica ...okay I will stop.

THIS IS ALL REVIEW

Hormone from Hypothalamus	Hormone from Ant Pit. and cell type	Hormone that goes to target organs and what these targets are	Inhibited By:	Actions	Diseases
GHRH	GH from somatotrope	GH (liver and peripheral tissues)	Somato- statin	Stimulates production of IGF1 & somatomedins that stimulate growth plates. IGF 1 will then inhibit GHRH secretion and stimulate somatostatin release.	Dwarfism in kids, Gigantism in kids and acromegaly in adults who will have thick hands, feet and wide face
Seems like TRH	TSH	Prolactin (Mammary glands, ovary, uterus)	Dopamine <i>Stimulated</i> by estrogen and	Breast development, milk production,	Both hypo and hyperprolactinemia causes infertility. Excess PRL is galactorrhea

			suckling mechanism	inhibits ovulation (decreased fertility during breast feeding)	
TRH	TSH from thyrotropes	TH (every organ you got)	Somatostatin (so just to review this puppy inhibits GH, glucagons, insulin, GI hormones and TH)	Controls BMR(basal metabolic rate), CNS (esp during prenatal), Cardiovas. system	Hyperthyroidism – sweating, increased CO Hypo – hypothermia, myxoderma (decreased Body Temp and lack of energy)
GnRH	FSH and LH (gonadotrophins)	Estrogen, testosterone or progesterone (gonads)	Inhibin and testosterone inhibit; low levels of estrogen inhibit while high levels of estrogen causes Positive feedback and LH surge	Reproduction	Lack of – delayed puberty, amenorrhea (irregular periods) and infertility Too much – Hirsutism which means lots of body hair (not going to mention any possible candidates here.☺)
CRH	ACTH from corticotrophins	Glucocorticoids like cortisol (adrenal gland to make glucocorticoids)	Cortisol involved in negative feedback	Stress reponse, metabolism	Lack of ACTH – hypotension, weakness Too much – Cushings Disease (vs Cushings Syndrome where ACTH is decreased but have increases cortisol levels), hypertension, moon face

Side notes - Oxytocin and Vasopressin are made in Hypothalamus and stored in Posterior Pituitary and in the middle of Pituitary gland (pars intermedia) – MSH for pigment production

NEW SHIZZNIT

-- Combined Pituitary Hormone Deficiency is the lack of GH, PRL, TSH caused by single mutation in Pit-1 gene (**remember without P I T no Prolactin, (okay to remember this next one just look at a capital I – it stands tall – height – GH) or TSH** Yes it's cheesy but I bet none of you will forget it ☺)

-- Pit 1 is needed for normal differentiation of somatotropes, lactotropes, and thyrotropes. Corticotrophs and Gonadotrophs are fine.

-- For differentiation of pituitary cells, need Pit 1 and GATA 2 TFs.

Just Pit 1- get lactotrophs and somatotrophs (PRL AND GH)

Pit 1 and GATA 2 – get thyrotropes (TH)

Just GATA 2 – gonadotrophs (usually Pit 1 inhibits this)

It seems like from the charts corticotrophs need NeuroDi and beta 2 so it doesn't follow this pattern.

-- There are two types of Pit 1 mutations – differ in phenotype of heterozygous (Hh) patients.

-- Hh patients carrying dominant mutation will develop Combined Pit. Hormone Def.

-- Hh patients carrying recessive mutation will be just healthy carriers while homozygous patients will only develop disease.

-- Board symptoms of Pituitary Tumors – just know general stuff like if one is having problems with sexual drive or decreased periods – probably problems with Gonadotrophins.

MOST common tumor is Prolactinomas – not sure why yet
Second most common are Null Cell Adenomas meaning tumors with no function.

SYNDROMES OF MULTIPLE ENDOCRINE NEOPLASIA(MEN)	
MEN-Type 1	MEN-Type 2
<u>Pituitary tumors</u>	<i>MEN-2a and -2b</i>
Eosinophilic adenoma (acromegaly)	<u>Medullary carcinoma of the thyroid</u>
Prolactinoma	<u>Pheochromocytoma</u>
Nonfunctional tumors	
ACTH-secreting tumors	<i>MEN-2a</i>
	<u>Hyperparathyroidism</u>
<u>Hyperparathyroidism</u> (Excess PTH)	<i>MEN-2b</i>
<u>Pancreatic tumors</u>	<u>Mucosal neuromas</u>
<i>Most common</i>	Marfanoid habitus
Gastrinoma	typical facies
Insulinoma	Bowel abnormalities
Pancreatic polypeptide-secreting tumor	
<i>Uncommon</i>	
Glucagonoma	
VIPoma	
GRFoma	
	Abbreviations: ACTH, adrenocorticotropic hormone; MEN-2a, -2b, multiple endocrine neoplasia types 2a, 2b.

MEN stands for Multiple Endocrine Neoplasia
To remember this chart:

-- MEN 1 – PPP – Pituitary tumors, parathyroidism and Pancreas

So will see tumors like Prolactinoma, excess PTH and problems associated with Pancreas

-- MEN 2 a – TPP – Thyroid carcinomas, Parathyroid and Pheochromocytoma (this means tumor of adrenal medulla so problems with SNS, epi or norepi)

-- MEN 2b – instead of hyperparathyroidism – see mucosal neuromas

*** MEN 1 – mutation in Tumor suppressor gene called Menin suppressor gene while MEN 2 is mutation in proto oncogene RET.

Remember that either activating mutations of an oncogene or inactivating mutations of a tumor suppressor gene results in genetic tumor-prone syndromes. Generally, proto-oncogenes, in normal cells are involved in promoting proliferation or suppressing cell death. A mutation that activates an oncogene generates a hyperactive protein, which acts dominantly over a normal protein and induces cancer development by increasing cell proliferation.

Tumor suppressor genes (TSG) in normal cells play roles in inhibiting cell proliferation or activating cell death. If there is a loss of one allele, you still have the other allele to inhibit cell growth and cause cell death. However, complete loss of the function of a tumor suppressor gene, which requires alterations in **both alleles**, leading to cancer development. There are **exceptions** to this and don't confuse the idea that TSG's are recessive at the cellular level but can be associated with **Autosomal Dominant syndromes**.

SO MEN 2 is an autosomal dominant mutation in proto oncogene RET – an activating mutation.

However MEN 1 is an autosomal dominant mutation in the tumor suppressor gene too – there are 2 possibilities for this.

- 1 To show this autosomal dominance, the mutant protein can interfere with WT protein and function as a dominant negative mutant
2. Or the Two Hit hypothesis where you are already a carrier and this doesn't cause tumors **but** later on, you get a somatic mutation and you lose your WT allele – this leads to **loss of heterozygosity (LOH)**.

Cancer Lectures (Both Lectures)

--An important point is that cancer is not inherited but its tendency is.

--Some definitions:

Somatic mutations aren't transmitted to offspring.

Sporadic mutations are mutations you gather during life that lead to cancers.

Familial – first mutation inherited. This then increases the tendency for a person to get a second mutation and get cancer.

Oncogenes and Tumor Suppressor genes – explained above.

--To review, LOH is loss of WT allele in the heterozygous carrier resulting in the cancer genotype. The second mutation is a somatic mutation caused by the environment. Only one cell requires the second somatic mutation.

Two hit hypothesis – e.g. in retinoblastoma

Sporadic retinoblastoma requires two somatic mutations however, in hereditary retinoblastoma, one mutation has already been acquired thru the germ line and therefore, they only need one more somatic mutation.

Other genes that predispose you to cancer include metabolic detoxification where you have increased susceptibility to mutagens, genetic instability or mutations in DNA repair.

Environment does not cause cancer but causes mutations – radiation, chemical carcinogens (no Henish – cell phones weren't listed as one possibility)

TSGs

1. - Retinoblastoma (RB1) – autosomal dominant. RB1 inactivation causes loss of heterozygosity and then a second mutation inactivates the RB1 gene. RB acts as TSG and inhibits progression past the restriction point in G1 (growth phase).

2. p53 – mutations in p53 are either mostly sporadic and are rarely familial. Li-Fraumeni syndrome is a p53 familial cancer where the protein translated from the mutated allele may function as a dominant negative mutant, leading to autosomal dominance. P53 is required for apoptosis and loss of p53 can lead to cell immortalization.

3. APC (adenomatous polyposis coli) – this is a gene name and a syndrome name (syndrome can also be called Familial Adenomatous Polyposis). APC is often mutated in colon cancers. It regulates cell adhesion, cell migration and apoptosis in colon crypts.

Cancer is a multistep process where both the activation of oncogenes and the inactivation of tumor suppressor genes are critical.

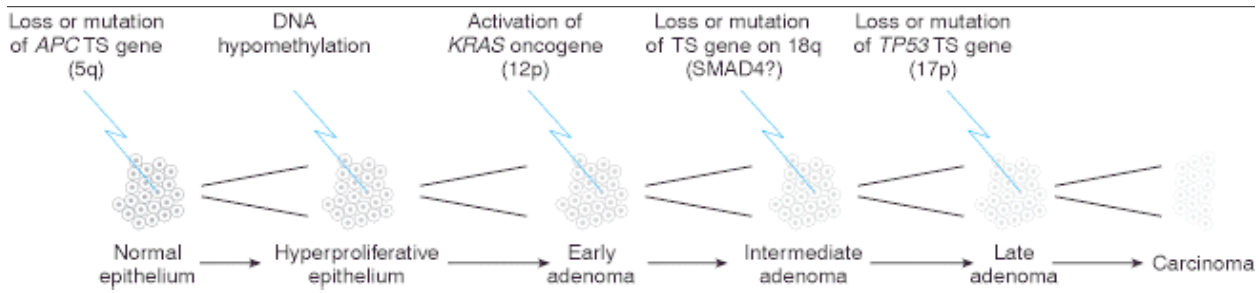
For example, in colon carcinomas:

1. Loss of APC TSG (leads to cell proliferation)

2. Activation of kRAS oncogene (early adenoma)

3. Loss of MADR2 TSG (also called 18q SMAD4 gene later in packet) (late adenoma)

4. Loss of p53 TSG (carcinoma)



Factors in hereditary cancer

1. Penetrance is the fraction of individuals with a certain genotype that manifest the phenotype.
 2. Expressivity is the **range** of phenotypes manifested by individuals with that particular genotype.
 3. Nature of mutation – involves the number of genes involved
- Other factors are hot spots for mutations such as p53 gene.

GENE	PHENOTYPE	PENETRANCE
TUMOR SUPPRESSOR GENES	Tumor initiation after second mutation	80-90%
GENETIC INSTABILITY OR DNA REPAIR	Increased mutational rate	50-70%
METABOLIC GENES	Detoxification of mutagens	<10-20%
ONCOGENES	*lethal in germline	0

- Some of the general features of hereditary cancer syndromes are:
- Same or linked forms of cancer in two or more close relatives
 - Earlier than usual cancer onset in one or more relatives
 - Bilateral cancer in paired organs such as in breast cancer both breasts develop cancer
 - Multiple primary tumors in the same individual
 - Evidence of autosomal dominant transmission of cancer susceptibility

Now I will just add some more info on these familial cancers but I have gone through most of them already...

1. Retinoblastoma

- Bilateral tumors usually diagnosed at 12 months with onset of tumors at 18 months
- Again, hereditary retinoblastoma is autosomal dominant,
- Two hit hypothesis applies here.

2. Colorectal cancers

- Most are sporadic but there are some familial cases –
 - a. FAP or APC (familial adenomatous polyposis) – colon has thousands of polyps that aren't malignant but if left alone, can evolve into cancer. Gene responsible is APC
 - b. Hereditary non-polyposis colon cancer (HNPCC) is also autosomal dominant but unlike FAP, there is no preceding phase of polyposis. Also, cells of HNPCC patients carry a nonfunctional DNA MMR allele (mismatch repair) – this causes accumulation of mutations at a high rate.

3. Breast cancers

- Familial breast cancers account for 5 to 10% of all breast cancer, and a substantial number of these cases are linked to mutations in the genes BRCA1 or BRCA2. These two genes have also been linked to most of the 8% of familial ovarian cancers.
- The transmission of the heritable susceptibility follows an autosomal dominant pattern. It is estimated that carriers have a 50 to 85% chance of developing breast cancer by the age of 70.
- BRCA1 and 2 are important for the pathway that protects cells from the effects of DNA damage. It is thought that BRCA1 and BRCA2 behave like classic tumor suppressor genes, with the loss of one copy predisposing the carrier to the development of the characteristic cancers.

4. Li-Fraumeni syndrome

- Li-Fraumeni syndrome (LFS) is autosomal dominant and occur due to mutations in the p53 gene for about 70% of classical LFS families, but p53 has been ruled out in some classic families, suggesting that this syndrome is genetically heterogeneous.
- The tumor spectrum in LFS is SBLA (Sarcoma, Breast, brain tumors, Leukemia, lymphoma, laryngeal carcinoma, lung cancer, Adrenal cortical carcinoma). Melanoma, germ cell tumor, and prostate carcinomas have also been described in LFS.

Structural Gene Mutations

- Osteogenesis Imperfecta
 - Genetic Properties
 - Autosomal Dominant
 - Both genders affected
 - Expressed in heterozygous and homozygous
 - Given to 50% of offspring
 - Chromosome: depends on type
 - Gene: COL1A
 - Protein: Collagen
 - Location:
 - Type: Loss Of Function
 - Parent Bias:
 - Classes of disease and associated symptoms
 - Type I
 - Childhood fractures, Blue sclerae, Normal teeth, Normal stature
 - Type II
 - Lethal, Congenital fractures, Respiratory insufficiency
 - Type III
 - Congenital fractures, Variable Blue sclerae, Abnormal teeth, Short stature
 - Type IV
 - Bone fragility, Variable Grayish sclerae, Abnormal teeth, Short stature
 - Symptoms
 - Hyperextension, thin skin, herniation likely, and above
 - Modes Of Genetic Transmission
 - Nonpenetrance
 - The expression of the disorder skips a generation
 - Somatic Mosaicism
 - New mutation during embryonic development causes a mixture of cells with or without the mutation. If germ-line cells affected, then passed onto kids
 - Molecular Basis
 - Collagen has a very long triple helix = 2 X alpha1 + 1 X alpha2
 - The 3 procollagen made in the ER form the triple helix in the ER
 - Modified and glycosylated
 - Transported to outside
 - N and C terminals cleaved
 - Protein Suicide Model
 - a/k/a Dominant Negative Effect
 - If one of the procollagen chains – 3 in the triple helix of one collagen molecule – is messed up then the cell degrades the final product before secretion, so only about 25% of the collagen made in an OI affected cell ever gets out
 - The collagen that gets out of the cell is normal but is made in very small amounts, hence the disorder
 - In the case in the handout
 - An alpha1 chain is found which is twice normal size, it is a dimer, has a diS bond between two cysteine residues in the procollagen
 - Suggested that the kid had a Gly – Cys substitution in COL1A1 gene

- Detection
 - Chorionic Villus Sampling can be used for detection during fetal development
 - RFLP
 - Restriction Fragment Length Polymorphism
 - Genes have polymorphisms in the noncoding regions which are often different in all people. Inherited from mom and dad. Like fingerprints of DNA. Used in Paternity testing.
 - In RFLP, cut the gene with a known restriction enzyme that produces known fragments, Run Southern Blot to see fragments of control and experiment on a gel. Use an appropriate probe
 - Apparently in one family tree, the presence of a polymorphic site in one of the COL1A genes leads to expression of OI
- Mouse Model
 - Clone the gene
 - Cut out a small piece of importance with restriction enzymes
 - Replace with synthetic oligonucleotide with point mutation
 - Inject into fertilized mouse oocyte
 - Grab your transgenic rodent
 - Make sure you have the right mouse by testing its protein panel for the messed up collagen and check for the messed up RNA transcript and DNA for the point mutation
- Osteoporosis
 - Also has messed up COL1A1 gene
 - G-T mutation in first intron
 - Don't care about the other syndromes with bad collagen
 - If you're getting your panties in a bunch about this then go put on a fresh pair and read the fucking table in the fucking handout
- Marfan Syndrome
 - Genetic Properties
 - Autosomal Dominant
 - Both genders affected
 - Expressed in heterozygous and homozygous
 - Given to 50% of offspring
 - Chromosome: depends on type
 - Gene: FBN-1
 - Protein: Fibrillin
 - Location:
 - Type: Loss Of Function
 - Parent Bias:
 - Symptoms
 - Very tall Stature, Long fingers
 - Easily displaceable joints
 - Cardiovascular problems
 - Aortic stenoses likely with possible aneurysm
 - Mitral Valve Prolapse
 - Heart murmurs
 - Dislocation of lens of eye
 - Stretch marks on skin without effort or weight change
 - Modes Of Genetic Transmission
 - Dominant Negative Effect
 - Molecular Basis
 - G-T transversion
 - Causes Glu to stop codon mutation, so early truncation
 - GAA to TAA
 - Detection
 - Immunofluorescence staining of fibrillin from skin fibroblasts
 - Blood sample DNA analysis to find mutation in FBN-1 gene
 - Chorionic Villus Sampling can be used for detection during fetal development

- Duchenne Muscular Dystrophy (DMD)
 - Genetic Properties
 - X-Linked Recessive
 - Males more likely to express
 - Expressed in hemizygous (XY) males and homozygous recessive females
 - Heterozygous females are carriers
 - No male to male transmission
 - Given to 50% of male offspring by carrier female
 - Affected male makes all female offspring carriers
 - Affected female gives it to all male offspring and makes all female offspring carriers
 - Chromosome: X
 - Gene: DMD
 - Protein: Dystrophin
 - Location: exon 51
 - Type: Loss Of Function
 - Parent Bias:
 - Classes
 - A milder form of this disorder is called Becker Muscular Dystrophy
 - Has abnormal quantity or quality of Dystrophin
 - Often found to have lesser deletions or mutations in BMD
 - Symptoms
 - Respiratory infections
 - Patient eventually becomes wheelchair bound
 - Serum creatine phosphokinase (CPK) level very high, normal is <30 mU/mL
 - Muscle weakness
 - Difficulty climbing stairs
 - Impaired motor control, clumsiness
 - Mental impairment
 - Detection
 - Carrier status can be tentatively determined by CPK level test
 - Chorionic Villus Sampling can be used for detection during fetal development
 - Fluorescence activated chromosome sorting
 - X marked with fluorescent marker
 - Laser causes marker to light up
 - Detector sees light causes that fluid drop with X to have a certain charge
 - This drop is separated from rest by magnetic field
 - DNA analysis and Southern blotting can be used to detect polymorphism which can separate normal and interrupted DMD gene
 - Immunofluorescent Dystrophin Staining
 - Good test for DMD and BMD differentiation
 - If no Dystrophin is present then DMD
 - If an abnormal quantity or quality of Dystrophin is present then BMD
 - Modes Of Genetic Transmission
 - X Inactivation
 - Mary Lyon's experiments found the inactivated X chromosome in mice
 - Lyon Hypothesis
 - ✧ She said that one of the two X chromosomes in each cell derived from the parents is randomly turned off during zygotic development
 - ✧ Produces a mosaic of cells that have one or the other X active, giving progeny which demonstrate the same inactivated X
 - Can also be demonstrated by glucose-6-phosphate dehydrogenase polymorphism in females
 - ✧ Cells in a female with two different polymorphs of G6PD will demonstrate one or the other polymorph
 - On the long arm of X immediately under the centromere is the X inactivation center Xic
 - The Xist gene is the actual culprit that causes the methylation on the X chromosome
 - ✧ This is a geeky mnemonic but think Xist-tentialism, I'm not good with mnemonics alright. Shut up Henish and Wisser.
 - DMD gene is flanked on one side by L128 and on the other side by D2
 - This helped in mapping of gene when there is a deletion or translocation which interrupts the p21 band which is where DMD gene is

- They found some poor bastard with a whole bunch of messed up shit but his haplotype showed that he only had a deletion around the p21 band which is where the DMD gene is so that was proof positive of the location of the culprit
 - The stupid ass restriction fragments can go to hell on their gel for all I care. Again questions, concerns? Put on a fresh pair of undies and read the fucking handout
 - The page about the XXXXY male is also retarded and not worth it
 - Molecular Basis
 - Methylation
 - Pattern is different for most people
 - Is inherited from parent
 - Newly replicated DNA strand undergoes methylation identical to template strand
 - The C next to G are methylated at 5' end of genes
 - Hypermethylation causes transcription inactivation, like in Fragile X Syndrome
 - Dystrophin
 - Product of DMD gene
 - Alpha helix in the middle
 - One side has an actin-binding domain
 - Other side has Cys rich Ca-binding domain and at the end a C terminal protein binding domain
 - In DMD exon 51 is often missing
 - Here's how all the shit connects up in the cell
 - Actin -> actin-BD on Dystrophin -> C-term PBD -> Dystrobrevin and Cys region -> Sarcoglycan region -> Dystroglycan complex -> Laminin-2
 - Bad Dystrophin
 - ◇ DMD and Becker Muscular Dystrophy
 - Bad Sarcoglycan Complex
 - ◇ Limb Girdle Muscular Dystrophy
 - Bad Laminin alpha-2 chain
 - ◇ Congenital Muscular Dystrophy
 - Treatment
 - Injecting normal myoblasts into dystrophic muscle
 - Dystrophin expression reasserted via gene therapy

Lectures 39 &40: Immunogenetics

MHC/HLA

Evolution of MHC complex

1. Immunoglobulin-like domain
2. Gene duplication
3. Gene conversion
4. Point mutation (non-synchronous>synchronous)
5. Recombination

Class I MHC

- on all nucleated cells
- present endogenous peptide
- α -subunit with four domains – α 1 and α 2 make up the peptide binding groove, α 3, and a transmembrane domain
- β -2-microglobulin – a stabilizing subunit
- forms synapse with Ag and TCR from CD8⁺ T cell
- α subunit is of the HLA-A, HLA-B, or HLA-C type
- highly polymorphic (~400 alleles for the A,B, and C combined)

Class II MHC

- on APCs only
- present exogenous peptide
- two subunits, α and β , both of which have three domains (two extracellular, plus a transmembrane domain)
- the first domains of each subunit make up the peptide binding groove
- forms synapse with Ag and TCR from CD4⁺ T cell
- of the type HLA-DR, HLA-DQ or HLA-DP

- highly polymorphic (~400 alleles for DR, DQ, and DP combined)
- DR α only hooks up with DR β , not DQ β or DP β , but each is highly polymorphic
- non-random association of α and β subunits - a preference called linkage disequilibrium
- Certain MHC allelic combinations may provide an advantageous view of the antigenic universe for populations in particular environmental settings (e.g. humans in the malarial belt). Conversely they may create unfortunate blind spots and a hasty diminution of the involved alleles as long as an offending pathogen continues to stalk the population.

MHC gene complex

- on chromosome 6
- divided into 3 domains
- contains a lot of different genes
- highest rates of polymorphism in human genome at MHC peptide presenting loci
- “The high level of MHC polymorphisms presumably provides a protective advantage for resistance to diverse infections”

Major functions of key loci within MHC

- Class I: Ag presentation to CD8+ T cells
Downregulation of NK killing
- Class II: Ag presentation to CD4+ T cells
Ag processing (TAP1/2,LMP2/7)
- Class III: Bf, complement pathway (C2,C4)
Regulatory cytokines (TNF α /TNF β)
Ag transport (HSP70)

Implications of MHC antigen presentation

- MHC molecules govern all specific adaptive immune responses
- An antigen is a peptide fragment that is processed and presented by a particular restriction MHC molecule (MHC)
- All proteins, foreign or self, contain potential antigenic epitopes (risk for autoimmunity)
- The observed high levels of MHC polymorphism reflect overdominant expression (heterozygous advantage)

HLA association with infectious diseases (Malaria, TB, Leprosy, HIV, Hep B, Hep C) some HLA variants provide resistance to certain diseases, some susceptibility

also strong correlations between HLA variants and autoimmune diseases, including: INSULIN DEPENDANT DIABETES MELLITUS, narcolepsy, rheumatoid arthritis, ankylosing spondylitis, Hodgkin’s, psoriasis, multiple sclerosis

for diabetes DQ3.2 is susceptible (like Dairy Queen and Baskin Robbins(32 flavors) – makes you sick), DQ3.3 is resistant (Dairy Queen alone is okay)

Remember from Immuno:

- TCR rearrangement
 - α and β chain ($\delta\gamma$ also possible)
 - VJ rearrangement on α chain
 - VDJ rearrangement on β chain
- Immune Synapse
 - T-cell with TCR
 - Antigen (Ag)
 - APC (or any other cell) with MHC

Transplant Stuff

Types of transplants

- Autograft: tissue removed then grafted to same individual (Donor=Recipient)
- Isograft/syngeneic graft: donor and recipient genetically identical (i.e. identical twins)
- Allograft: from one individual to a different individual within species
- Xenograft: from one species to another

Sites of Transplants

- orthotopic (organ transplanted to usual anatomic site)
- heterotopic (organ transplanted to atypical anatomic site)

Tissue Compatibility Barriers

- Graft rejection (host versus graft reactions (HvG))
 - Snell's Dictum: "Isografts take, allografts fail"
 - Hyperacute (minutes to hours)
 - Acute rejection: mismatch leads to sensitization, 2-14 days
 - Chronic rejection: slow rejection months after transplant
- Graft versus host reaction (GvH)
 - Billingham's rules for GVH reactions
 - Genetic disparity
 - Immuno-competent donor cells
 - Immuno-compromised recipient

Minor Histocompatibility Antigens – ‘background’ genetic differences (non MHC) which contribute to graft vs. host and host vs. graft diseases
 -i.e. contribute to low grade and delayed transplant rejection
 -that is, the MHC matches, but the peptide presented is viewed as foreign

HLA matching

- if host has all the graft HLA, plus a different HLA
 get graft vs. host, because graft sees something different, but host doesn't
- if graft has all the host HLA, plus a different HLA
 get host vs. graft, because host sees something different, but graft doesn't

Graft vs. Host disease

- pretty much always bad
- BUT, kills cancer cells too, so . . . lower rates of relapse for leukemia patients

If transplant hematopoietic stem cells, then get graft vs. host disease. However, this tapers off with time (~6 months), at that time, the old, host-reactive T-cells are dead, and new host thymus educated cells are out in full force. Immunosuppression can be stopped. ALSO – you can then transplant other tissues from the donor with out rejection. Cool.

Targets of graft vs. host disease

- skin, liver, gut, immune system, hematopoietic system