David Bejar
BHDII/Hematology & Oncology
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Hematology/Oncology Study Guide

Lecture Reviews

Lecture #1: 11/16- Iron Metabolism

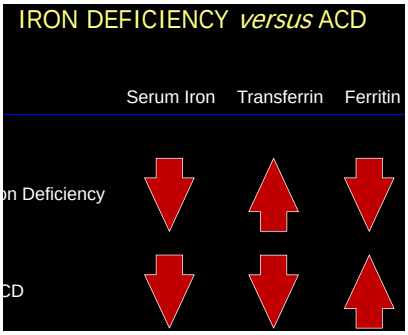
- Red Blood Cell
  - Size: similar to the **nucleus of a lymphocyte**

RBC Parameters	Normal Values
Hemoglobin	
Females	12.0-16.0 gm/dl
Males	13.5-17.5 gm/dl
RBC Count	
Females	4-5.2x10^{12}/l
Males	4.3-5.6 x 10^{12}/l
MCV	80-100 fl
Reticulocyte Count	0.2-2.0%
WBC	4-10 x10^{9}/l
Platelets	150-400 x10^{9}/l

- Anemia
  - Definition
    - **D*ecrease in number of circulating RBCs***
    - Most common hematologic disorder by far
  - Causes
    - Blood loss, decreased production (marrow failure), increased destruction (hemolysis)
    - **Reticulocyte Count**
      - Decreased production: **decreased**
      - Increased destruction: **increases**
      - Retic. Count = Retic % x RBC Count
      - Normal up to 100,000/uL
  - Decreased Production
    - **Iron**
      - Physiology
        - Must be in ferrous (2+) state for activity
        - Iron readily donated electrons to oxygen, creating **reactive oxygen species;** thus limiting exposure of body parts to iron is a major survival advantage

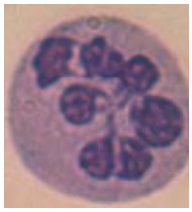
        - Majority of iron body is stored in **hemoglobin**
        - Transport:
            - Iron circulates in the body bound to **transferrin**
            - Upon binding to transferring receptor, the entire complex is endocytosed and **iron is released** intracellulary via **lysosomal acidification**
- **Iron Deficiency Anemia**
    - Causes:
        - Blood loss (GI, menses), increased iron utilization (**pregnancy**, polycythemia vera), malabsorption (gastrectomy, atrophic gastritis)
    - Progression:
        - Low serum ferritin→ desaturation of transferrin→ serum iron drops→ transferrin increases→ blood smear: **microcytic, hypochromic, aniso- and poikilocytosis**→ anemia
    - Symptoms
        - **Fatigue**, atrophic glossitis, koionychia (nail spooning), esophageal web
    - Therapy
        - Replacement
            - Oral (100-900 mg/day), requires acid environment (i.e. Vitamin C)
            - Initial response: takes 7-14 days
            - Correction of anemia: 2-3 months
            - 6 months therapy needed beyond correction
            - *Parenternal possible (allergic rxn!)*
- **Hemochromatosis**
    - General
        - Excess iron uptake
        - Seen especially amongst Northern Europeans
        - Genetic mutation in HFE gene
    - Pathogenesis
        - **Defect in HFE**→ decreased iron uptake by crypt GI cells→ **decreased intracellular iron**→ increases DMT-1→ **increased iron extraction from diet**→ increased iron delivery to tissues
    - Clinical
        - Iron deposition in tissues causes:
            - Skin darkening (+iron→+ melanin)
            - Endocrinopathy (DM, hypothyroid)
            - Liver damage (cirrhosis→ HCC)

        - Cardiomyopathy (→ CHF)
    - Treatment
        - Phlebotomy
        - Iron chelation (for transfusion-induced)
- **Anemia of Chronic Disease** (ACD)
    - General
        - Mild, non-progressive anemia
        - Usually **normocytic** (30% microcytic)
        - Normal reticulocyte count (inappropriately low for degree of anemia)
        - Most body iron in **STORAGE** compartment
    - Causes
        - Thyroid Dz, rheumatoid arthritis, systemic lupus erythematosus, IBD, malignancy, chronic infectious dz (TB, osteomyelitis)
    - Pathophysiology
        - **Hepcidin**
            - Upregulated in inflammatory/infectious states; down-regulated in iron deficiency
            - Induced **internalization of ferroportin**, leading to **decreased iron uptake from GI tract**
            - Part of cause of decreases iron absorption in ACD



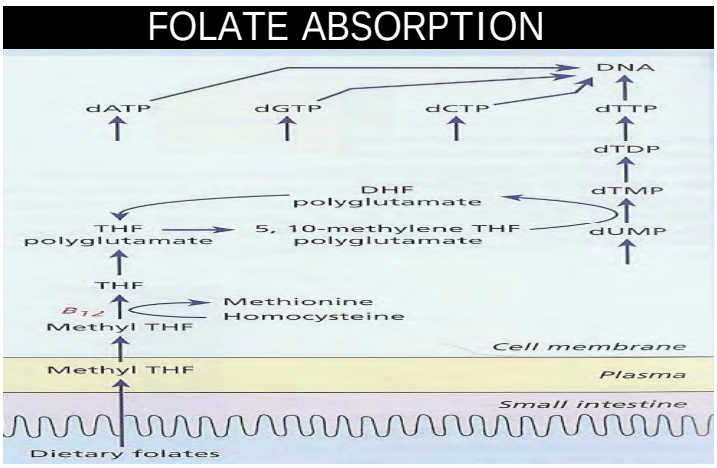
Lecture #2: 11/16- Megaloblastic Anemia

- Marrow failure: highly metabolically active, so any slowing of DNA production will lead to marrow failure
- Megaloblastic anemia is caused by a **defect in nuclear replication and division** which affects **all** marrow elements
- Hallmarks
    - **Oval macrocyte** (MCV > 100 fl)
    - **Hypersegmented Neutrophils** (see *fig*.)
    - Pancytopenia
    - Reticulocytopenia
    - LDH elevated
    - Serum iron normal or elevated
    - **Serum Vitamin B12 or Folate LOW!**
    - **Bone marrow**: **classic megaloblastic changes** (nuclear to cytoplasm dyssynchrony, which leads to cells being destroyed in marrow before reaching the circulation)



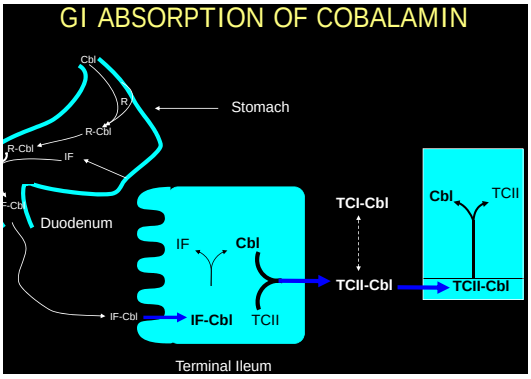
- Causes
    - Megaloblastic anemia caused by **folic acid** or **vitamin B12** deficiency
    - **Folic Acid**
        - Physiology
            - Necessary for thymine (DNA) biosynthesis
            - **Uptake of folic acid** in the small intestine is **facilitated by vitamin B12,** which allows for folate demethylation

and thus addition of a **polyglutamate tail** which traps folate in the cell



- Causes of Folate Deficiency
  - Folate-poor diet (**alcoholism**, poverty); rarely in US
  - Increased folate requirement (**pregnancy**, severe hemolytic anemia, psoriasis)
  - Chemotherapy (this is how chemo works)
  - Malabsorption (tropical sprue)

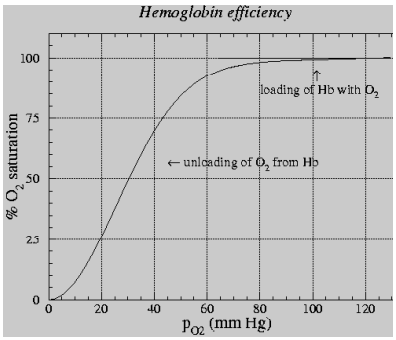
- **Vitamin B12 (Cobalamin)**
  - Physiology



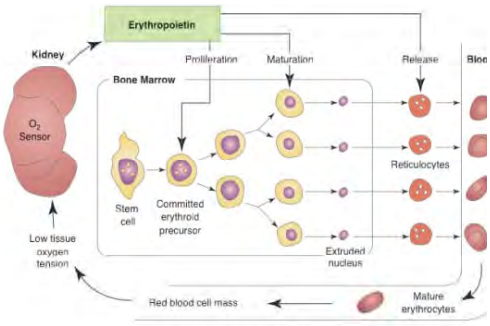
    - Necessary for: **demethylation of folate in GI tract**, methylation of myelin, conversion of methylmalonyl CoA to succinyl CoA (CAC)
    - Obtained from the diet; in the stomach, bound to **intrinsic factor** (IF) which protects VitB12 from digestive enzymes and allows for absorption into ileal enterocyte
    - In enterocyte, binds to **trans-cobalamin II** and is transported via circulation to hematopoietic precursor cell
  - Causes of Cobalamin Deficiency
    - Gastric failure (total gastrectomy, pernicious anemia)
    - Ileal failure (IBD, ileal resection, tropical sprue)
    - Competing organisms (bacterial overgrowth, *D. latum*)
    - **Pernicious Anemia**
      - Autoimmune, antibody-mediated destruction of parietal cells, which produce intrinsic factor
      - Achlorhydria universal
  - **NEVER CORRECT COBALAMIN (VitB12) DEFICIENCY WITH JUST FOLATE THERAPY BECAUSE, ALTHOUGH YOU WILL FIX ANEMIA, THE BRAIN ALSO REQUIRES COBALAMIN**
    - Neurological manifestations of cobalamin deficiency: dementia, psychiatric distubance, demyelinating dz

(**subacute combined degeneration** of the posterior columns of the spinal cord)
        - There is up to a 10 year storage pool of cobalamin in the blood, so it may takes year after onset of cobalamin deficiency for symptoms to appear
- Diagnosis and Treatment of Megaloblastic Anemia
    - Diagnosis
        - Draw blood levels before therapy to determine deficient substance (i.e. folate or cobalamin)
    - Treatment
        - Once levels draw:
            - **Treat with <u>BOTH</u> VitB12 *and* Folate**
        - Once levels back:
            - Can stop normal vitamin
        - Response to therapy is usually rapid and dramatic
    - Note:
        - AVOID TRANSFUSIONS unless patient in hemodynamic compromise and this can precipitate heart failure 2º to volume-overload

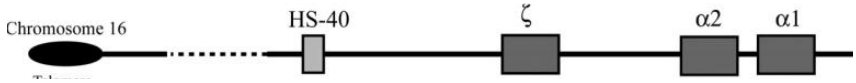
Lecture #3: 11/17- Hemoglobinopathies



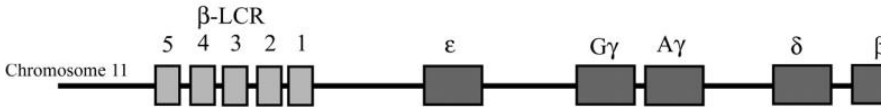
- **Hemoglobin (Hb)**
    - Tetramer: 2 alpha (A1, A2) and 2 beta (B1, B2) chains
    - Each chain contains a heme group + ferrous iron
    - Ferrous iron binds to oxygen
    - Upon binding to oxygen, conformational change occurs which increases Hb's affinity for oxygen
    - Oxygen dissociation: at PO2 <= 40 mmHg, there is significant **unloading** of oxygen (fig. oxygen dissociation curve)
- **Erythropoiesis**
    - When kidney senses low tissue oxygen tension, it produces and secretes **erythropoietin**, which travels to the bone marrow
    - At the bone marrow, erythropoietin stimulates 1) <u>proliferation</u> and 2) <u>maturation</u> of RBC precursors (nuclei extruded)
    - **Reticulocytes** (still contain some mRNA) are released from the bone marrow into the circulation and become mature RBCs



- **Globin Genes**
    - Globins
        - Gamma
            - Chromosome 11 contains 2 gamma genes
            - Active throughout <u>fetal</u> life ONLY



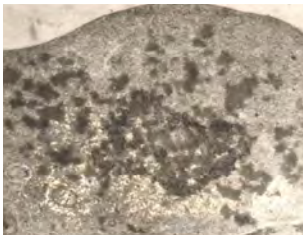
- **Alpha**
  - Chromosome 16 contains 2 alpha genes, A1 and A2 (1 Chr.16 x 2 parents = 4 total alpha genes)
  - Active throughout fetal *and* adult life
- **Beta**

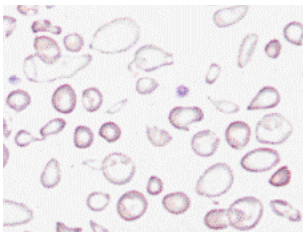


  - Chromosome 11 contains a single beta gene (1 Chr. 11 x 2 parents = 2 total beta genes)
  - Active throughout adult life ONLY

- Transcriptional Regulation
  - **Beta Locus Control Region (B-LCR)**
    - Essential for regulation of switch from fetal to adult globin genes
    - Translocation of B-LCR from gamma gene regulatory position to beta gene regulatory position allows for *intrachromosomal* ***looping out*** of gamma (fetal) genes

- **Thalassemias**
  - **QUANTITATIVE** change: decreased or absent production of a globin chain
  - **Beta Thalassemia**
    - Decrease in production of otherwise normal beta globin gene
    - Beta Thalassemia Homozygotes
      - **B^+ thalassemia**: 10-30% of normal B chain production
      - **B^0 thalassemia**: absent B chain production
    - Anemia results from decreased beta globin chain which leads to decreased **Hemoglobin A** (most common Hb, i.e. A2B2)
    - **Excess alpha globin** form **aggregates** which lead to RBC death and hemolysis in bone marrow (see *fig.*)
    - Blood Smear:
      - **Translucent RBCs** due to lack of Hb; **poikilocytosis**

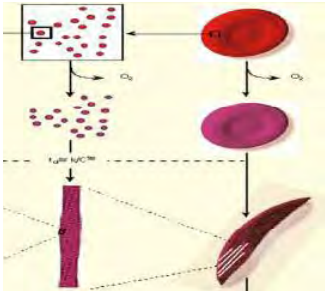




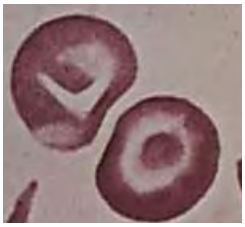
    - **Beta Thalassemia Trait**
      - Heterozygotes for beta thalassemia mutation
      - Develop only **mild** anemia
      - Mean corpuscular volume (MCV) is **LOW**!
      - *Low MCV w/ near normal Hb = Beta Thalassemia* ***Trait****!*
    - Treatment:
      - **Blood transfusions**
        - Problem: **iron overload**; body has difficult time excreting iron and thus iron deposits throughout body; deposition in heart leads to **cardiomyopathy**→ heart failure (leading cause of death in patients receiving regular blood transfusions for thalassemia); oral iron chelation therapy has proven helpful

- **Alpha Thalassemia**
  - Whereas beta thalassemias are usually point mutation affecting gene expression, alpha thalassemias are usuall whoe alpha globin gene deletions
  - Normally have 4 alpha globin gens (2 from each parent): If:
    - 3 alpha genes→ silent carrier
      - Asymptomatic
    - 2 alpha genes→ alpha thalassemia trait
      - Mild symptoms
    - 1 alpha gene→ HbH disease
      - Beta tetramer→ hemolytic anemia; manageable
    - 0 alpha genes→ hydrops fetalis (in utero death)

- **Hemoglobinopathies**
  - **<u>QUALITATIVE</u>** change: mutation in nucleotide sequence of globin gene produces abnormal globin structure
  - **Sickle Cell Anemia (HbS)**



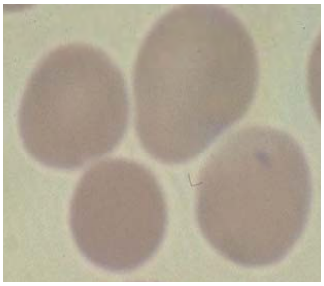
    - Mutation in 6th amino acid of beta chain ($B^{6\ glu \rightarrow val}$) produces abnormal Hb→ HbS
    - Sickling occurs when Hb unloads oxygen (i.e. in capillary bed)
      - HbS forms long polymers which distort the cytoskeletal architecture of the RBC, leading to sickling
    - Sickling is **reversible** when Hgb is once again reoxygenated; however, with continuous sickling, RBC **membrane is stressed** and **<u>HEMOLYSIS</u>** ensues→ hemolytic anemia
    - Sickling also leads to RBS aggregation and occlusion of capillaries→ **ischemia**→ **PAIN** crisis!
    - Intravascular hemolysis **consumes nitric oxide**, which leads to other complications of sickle cell disease:
      - 1) **Nonhemorrhagic stroke**
      - 2) Pulonary Hypertension
      - 3) Priapism
      - 4) Leg Ulcerations
    - Diagnosis:
      - 1) Hemoglobin electrophoresis (showing SS)
      - 2) Blood smear (showing sickle cells)
    - Treatment:
      - 1) **Hydroxyurea**
        - Increases number of RBCs with protective **HbF** by decreasing the number of RBC precursor cell divisions, and thus shunting RBC production to younger lineage with HbF (NEJM Article)
      - 2) Transfusions
        - Problem: iron overload
      - 3) Bone Marrow Transplantation
        - Younger age at transplant associated with fewer complications



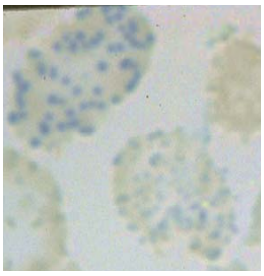
- **HbC**
  - Mutation in 6th amino acid of beta chain ($B^{6\ glu \rightarrow lys}$) produces abnormal Hb→ HbC
  - See **target cells** on blood smear (see *fig*.)
  - Anemia is less severe than sickle cell anemia
  - Can have Sickle-HbC Disease (SC)

Lecture #4: 11/17- Hemolytic Anemias

- Hemolytic anemia is an anemia of **increased RBC destruction**
  - Normochromic
  - Shortened RBC survival
  - Reticulocytosis
- Signs of Hemolysis
  - Serum conjugated bilirubin→ increased
  - Serum LDH→ increased
  - Serum haptoglobin (removes Hb from circulation)→ decreased
  - Urine Hb→ present
  - Urine urobilinogen→ increased
  - Reticulocyte count→ increased
- Blood Smear
  - **Normal blood smear is common finding in hemolytic anemia**! Therefore, normal blood smear DOES NOT exclude hemolytic anemia
- Intracorpuscular Hemolysis
  - Membrane Abnormalities
    - Microskeletal Defects



      - **Hereditary Spherocytosis**
        - Spectrin = major cytoskeletal protein that gives RBC its biconcave shape
        - **In hereditary spherocytosis, spectrin is defective or absent**
        - Loss of biconcave shape, seen as loss of central palor on blood smear (see *fig*.)
        - **Decreased deformability** and **increased osmotic fragility→ hemolysis in spleen**
    - Increased Sensitivity to Complement
      - **Paroxysmal Nocturnal Hemoglobinuria**
        - Ongoing intra- and extravascular hemolysis
        - Classically at **NIGHT**, when shallow breathing leads to decrease in pH which activates complement
        - Acquired **deficit of GPI-Associated protein** (inc. ***decay accelerating factor***) which deactivates complement-formed **membrane attack complex** on RBCs
        - Wake up with brown urine (bili)
        - Tests: CD59 and CD55 negative



- Enzymopathies
  - **Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency**
    - Glutathione (GSH) protects RBC from oxidative stress
    - G6PD regenerates NADPH, which regenerates GSH
    - **In G6PD Deficiency, you have increased levels of hemolysis during oxidative stress**
      - Infection, medications (nitroglycerin), fava beans
    - Oxidative stress leads to Heinz Body formation (see *fig*.)
- Extracorpuscular Hemolysis
  - Nonimmune
    - Mechanical
      - Vascular Abnormalities (no coagulation abnormality)
        - **Thrombotic Thrombocytopenic Purpura**
          - Classic Pentad (need first two)
            - **1) Microang. hemolytic anemia**
              - RBCs sheared by platelet plugs
            - **2) Thrombocytopenia**
            - 3) Mental status change
            - 4) Renal dysfunction
            - 5) Fever
          - Causes by absence of von Willebrand factor-**cleaving** metalloproteinase
          - Treatment:
            - 1) Plasmapharesis
            - 2) Steroids
            - 3) Splenectomy (for resistant dz)
  - Immune
    - All require antigen-antibody reaction
    - Antibodies combine with RBC and either 1) **activates complement** (intravascular lysis) or 2) **opsonize** RBC for immune system (extravascular lysis)
    - **Coomb's Test**
      - Looks for immunoglobulin +/- complement on RBC
      - Coomb's Reagent: mixture of anti-human immunoglobulin and anti-hunam complement
      - Mixed with patient's RBCs
        - If immunoglobulins or complement present on RBC surface, Coomb's reagents links cell together and causes agglutination or RBCs
    - Drug-Related Hemolysis
      - **Immune Complex Mechanism**
        - Drug and antibody bind in plasma
        - Immune complex activate complex or sit on RBC
        - RBCs lysed as innocent bystanders
        - Drugs: quinidine, quinine, isoniazid

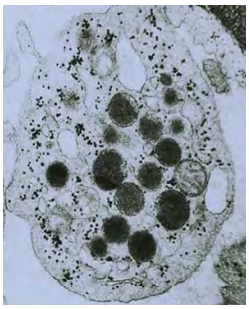
- **Hapteninc Mechanism**
    - Drug binds and reacts with RBC surface protein
    - Antibodies recognize altered protein as foreign
    - Antibodies begin hemolytic process
    - Drugs: penicillins, cephalosporins
- **True Autoantibody Formation**
    - Stimulate production of antibodies that react with antigens normally found on RBC surface
    - Drugs: Methyldopa, procainemaide, ibuprofen

- Alloimune Hemolysis
    - **Hemolytic Transfusion Reaction**
        - Caused by recognition of foreign antigens on transfused RBCs
        - Types:
            - **1) Immediate intravascular hemolysis (minutes): due to pre-formed Ab's (life threatening)**
            - 2) Slow extravascular hemolysis (days): due to repeat exposure to foreign antigen (mild)
            - 3) Delayed sensitization (weeks): due to 1st exposure to antigen (asymptomatic)
        - ABO and Rh testing must be preformed pre-transfusion to prevent this from occurring!
    - **Hemolytic Disease of the Newborn**
        - Due to incompatibility b/w mother negative for antgen and fetus/father positive for antigen
        - **ABO and Rh incompatibility** most common!
        - Usually occurs with **2nd or later pregnancies**
        - Can cause severe fetal anemia, with erythroblastosis and heart failure
        - Hyperbilirubinemia→ kernicterus (brain)
        - If due to Rh incompatibility, can be prevented by administration of ant-RhD to Rh negative mothers after each pregnancy
- Autoimmune Hemolysis
    - Oftten associated with lymphoproliferative disease or collagen vascular disease
    - Due to formation of autoantibodies that attack own RBC
    - **Warm Autoimmune Hemolysis**
        - IgG mediated; fix complement only to level of C3; complement recognized by macorphages, therefore hemolysis is extravascular; responsive to steroids
    - **Cold Autoimmune Hemolysis**

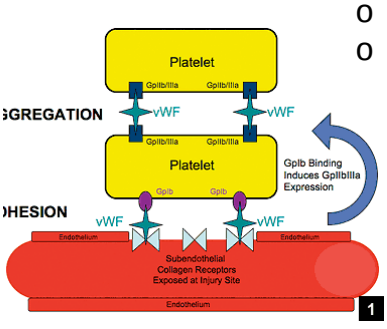
- IgM mediated; antibodies bind best at 30º or lower; fix entire complement cascade; leads for formation of membrane attack complex, therefore hemolysis in vasculature; poorly responsive to steroids; responsive to plasmapharesis

Lecture #5: 11/18- Hemostasis/Thrombosis I

- Hemostasis
  - **<u>Primary</u>: platelet plug formation**; dependent on normal platelet number and function
  - **<u>Secondary</u>: activation of clotting cascade**; deposition and stabilization of fibrin
  - **<u>Tertiary</u>: dissolution of fibrin clot**; dependent on *plasminogen* activation
- Bleeding disorders result from defects in **primary** or **secondary** hemostasis
- A **history** is critical for assessment of presence of a bleeding disorder (family history, heavy menses, easy bruising); however, **laboratory work** is essential for determining **specific defect** and **to monitor effects of therapy**



- **Bleeding time** = primary screening test for **platelet function**
  - Make nick in skin and record how long it takes for bleeding to stop
- Platelets
  - **Dense Granules**: contain calcium and serotonin (and ADP?)
  - **Alpha Granules**: contain protein mediators of platelet activation
  - Upon activation, platelets release contents of granules into circulation, which encourages platelet aggregation
- **Platelet Function Defects**
  - Clinical Manifestation: prolonged bleeding time
  - Platelet <u>Adhesion</u> Defects (Congenital)
    - **Bernard Soulier Disease**
      - Abnormal **GPIb-IX Complex**, which is **receptor for von Willebrand factor** (vWF) on platelet membrane
      - Test: ability to aggregate platelets in presence of **ristocetin** (will only occur if GPIb-IX present)
    - **Von Willebrand Disease**
      - *Later in lecture*



  - Platelet <u>Release</u> Defects (Congenital)
    - **Delta-Storage Pool Disease**
      - Failure to form **dense granules**
      - Thus, do not release ADP, Ca++, 5-HT on activation
      - Fail to recruit platelets for aggregation
    - **Gray Platelet Syndrome**
      - Failure of packaging of **alpha-granules**
      - Thus, do not release protein mediators of aggregation
      - Mild bleeding disorder
  - Platelet <u>Aggregation</u> Defects (Congenital)

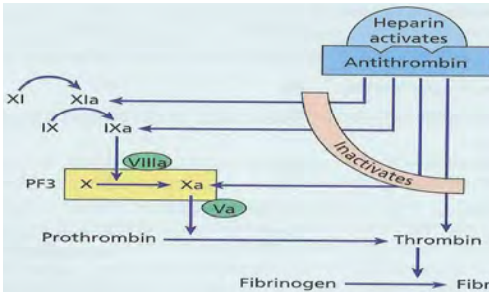
        - **Glanzmann's Thromboasthenia**
            - Autosomal recessive
            - Lack of **GPIIb/IIIa,** the fibrinogen receptor
            - Fibrinogen normally links platelets together via GPIIb/IIIa
            - Bleeding can be severe
        - **Scott Syndrome**
            - Defect in platelet microparticle formation
            - Loss of **shufflase**: platelets fail to produce receptor for Factors VIIIa and Va on surface of activated platelets
    - Acquired Drug-Induced Platelet Defects
        - Alcohol
        - Prostaglandin Synthetase (COX) Inhibitors (aspirin, NSAIDs)
        - ADP Receptor Inhibitors (clopidogrel, ticlopidine, prasugrel)
- **Thrombocytopenias**
    - Decreased Platelet Production
        - **Decreased megakaryocytes**
            - Platelets have **normal lifespan**
            - Good response to platelet transfusion
        - *Neoplastic diseases, primary marrow disorders*
    - Increased Platelet Destruction
        - Platelets have **shortened lifespan**, increased megakaryocytes, macroplatelets, poor response to platelet transfusion
        - Immune
            - **Idiopathic Thrombocytopenia Purpura (ITP)**
                - **IgG antibodies against platelets** and marrow
                - Platelets removed by macrophages
                - No good diagnostic test
            - **HIV-Associated Thrombocytopenia**
                - Early
                    - Immune-mediated
                    - In absence of AIDS!
                    - Marrow normal
                    - Treatment: **antiretrovirals**
                - Late
                    - Marrow infiltration
                    - Pancytopenia
                    - Treatment: poor response to all therapy
- Clotting Factor Disorders
    - Coagulation Testing
        - Methods:
            - Blood collected in sodium citrate, a calcium inhibitor→ blood won't clot spontaneously without calcium→ RBCs and platelets centrifuged off, leaving plasma
        - **Prothrombin Time (PT):**
            - Measures *extrinsic pathway*

        - Add tissue factor and Ca++ to plasma
        - Measure time to clot formation (N: 11-13 seconds)
        - Prothrombin (II)→ Thrombin (IIa)
        - Abnormal PT: check for factors II, V, VII, X
    - **Activated Partial Thromboplastic Time (aPTT):**
        - Measures *intrinsic* and *common coagulation pathways*
        - Add phospholipid and surface active agent to plasma
        - Measure time to clot formation (N: 25-35 seconds)
        - Abnormal aPTT: check for factors XII, XI, IX, VIII
    - If PT or aPTT abnormal, then perform:
        - Test for Missing Factor
            - For each assay, mix 50% patient plasma with 50% plasma deficiency in a single clotting factor
            - PTT/aPTT will normalize except when assayed with plasma devoid of missing factor, in which case clotting time will be prolonged
- **Von Willebrand Disease**
    - General
        - Mild bleeding disorder characterized by **lack of von Willebrand Factor**, which is normally synthesized in endothelial cells and stored in large multimers in Weibel-Palade bodies
        - Autosomal dominant; variable penetrance
    - Pathophysiology
        - Defective platelet adhesion
        - **Decreased factor VIII activity**
    - Clinical
        - **Bleeding Time:** prolonged
        - **aPTT**: prolonged
        - Ristocetin Cofactor Activity: decreased
    - Classification
        - Type I: quantitative defect
        - Type II: qualitative defect
        - Type III: **severe** quantitative defect
- **Hemophilia**
    - General
        - X-linked recessive
        - **Hemophilia A (85%): lack of factor VIII**
        - Hemophilia B (15%): lack of factor IX
        - A and B clinically indistinguishable
    - Clinical
        - **Mild** (5% factor level): bleeding and occasional hemarthroses only with significant trauma or surgery
        - **Moderate** (1-5% factor level): bleeding with mild trauma; hemarthroses with trauma, occasionally spontaneous

            - **Severe** (<1% factor level): spontaneous hemarthroses and soft tissue bleeding
            - **Bleeding time**: normal
            - **aPTT**: prolonged
        - Pathophysiology
            - Without factors VIII and IX, you can't make **tenase complex** (IXa-VIIIa), which is needed for the formation of **prothrombinase complex** (Xa-Va)
- **Factor XI Deficiency**
    - Ashkenazi Jews
    - Mild bleeding (typically only with procedures)
    - Rx: fresh frozen plasma
- Other clotting protein deficiencies
    - **Vitamin K Deficiency**
        - Hospitalized patients with **malnutrition** and **decrease in normal gut flora**
        - PT elevated
        - Treatment: vitamin K replacement
    - **Liver Disease**
        - Decreased synthesis of vitamin K dependent factors
        - Decreased platelets due to hypersplenism

Lecture #6: 11/18- Hemostasis/Thrombosis II

- **Anticoagulation Proteins/Complexes**
  - **Tissue Factor Pathway Inhibitor**
    - Complexes with TF-VIIa complex→ inactivated Xa (which forms prothrombinase complex, Xa-Va)
  - **Antithrombin III/Heparin Cofactor II/Heparin**
    - Heparin activates antithrombin III
    - Antithrombin III inactivates IXa, Xa, XIa, and IIa (thrombin) (*see figure*)
  - **Protein C/Protein S/Thrombomodulin**
    - Cleaves and inactivates VIIIa (tenase) and Va (prothrombinase)
    - In presence of thrombomodulin, thrombin loses ability to catalyze fibrinogen to fibrin
  - **Plasminogen (3º hemostasis)**
    - Cleaves fibrin



- Anticoagulant Protein Deficiencies
  - Heterozygous→ increased venous thrombosis, occasional arterial thrombosis
  - Homozygous→ neonatal purpura fulminans (NPF), fibrinogenolysis, chronic DIC
  - Dominant: venous thrombosis, young age, positive family history
  - Recessive: no history of thrombosis, no family history, NPF in offsping
- Inherited Hypercoagulable State
  - **Activated Protein C (APC) Resistance**
    - Hallmark: failure of activated Protein C to prolong aPTT
    - Mutation in Factor V: Arg 506→ Gln
      - Named Factor V Leiden
      - Found in >98% of patients with APC Resistance
      - APC cannot inactivated Factor **Va**, so you have increased prothrombinase activity (Xa-**Va**)
    - Increased risk of venous thromboembolism (heterozygous: 4x, homozygous: 10x)
- Acquired Hypercoagulable State
  - **Inflammatory Diseases**
    - **C4b Binding Protein**: acute phase reactant that is increases in inflammatory states
    - C4b binding protein **binds Protein S and inactivates it**
    - Inflammation also leads to downregulation of thrombomodulin
  - **Nephrotic Syndrome**
    - Excretion of large amounts of protein in urine, including:
      - Antithrombin III, Protein S, Protein C
      - C4b Binding Protein cannot fit through glomerular apparatus and so is markedly elevated in nephritic syndrome

- **Anticardiolipin Syndrome** (*Lupus Anticoagulant*)
  - Bleeding only in rare circumstances
  - Associated with **arterial and venous thrombosis**
  - Associated with **false positive RPR** (*Syphilis test*)
  - Associated with **recurrent spontaneous abortions**
  - Diagnosis: antiphospholipid antibodes that interfere with clotting process **in vitro**, NOT in vivo

- **Disseminated Intravascular Coagulation**
  - Acute DIC: *BLEEDING FORM*
    - Causes: shock, sepsis, mismatched transfusion, trauma, burns
    - Patients typically **bleed** due to **consumptive coagulopathy**
      - Decreases in both coagulants and anticogulants
    - Also see plasminogen activation, which functions in fibrinolysis
    - Rx: replacement therapy (FFP, platelets, cryoprecipitate)

DIC
Testing (Acute)

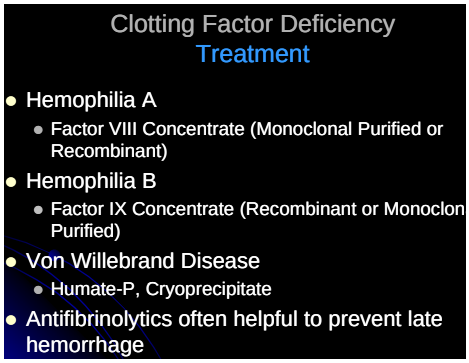
Test	Result
Prothrombin Time	Slightly to grossly prolonged
aPTT	Variable
Fibrinogen	Usually low
Thrombin time	Usually prolonged
Factor levels	Variable
Platelet Count	Usually low
RBC fragmentation	Sometimes present
Fibrin split products	Usually present

  - Chronic DIC: *THROMBOTIC FORM*
    - Causes: leukemia, carcinomas, aortic aneurysms
    - Patients usually have DVT, PE, arterial thrombotic events
    - Rx: anticoagulant therapy
  - **IN DIC, TREAT UNDERLYING DISEASE!**

Lecture #7: 11/19- Therapeutics: Coagulation and Anticoagulation

- Coagulation Therapy
  - Platelet Function Disorders
    - **DDAVP** (Desmopressin)
      - Increases plasma levels of factor VIII (involved in Xase complex which requires platelet phospholipid surface for proper functioning)
    - **Platelet Transfusion**
      - Only sure way to decrease bleeding
      - Should reserve for procedures only
    - **E-Aminocaproic Acid**
      - Directly inhibits the activation of plasminogen, thereby reducing conversion of plasminogen to plasmin, an enzyme that degrades fibrin clot; an antifibrinolytic
  - Thrombocytopenia
    - **Platelet Transfusion**
      - Given if <50,000/uL and undergoing invasive procedure
      - Given if <10,000/uL prophylactically
  - Idiopathic Thrombocytopenic Purpura (IgG destruction of platelets)
    - **Corticosteroids,** splenectomy, IV IgG
      - Inhibit macrophage clearance
    - **Rtuximab**

            - Destruction of antibody producing cells
        - **Eltrombopag, Romiplostim**
            - Thrombopoietin-mimetics
        - **Antiretroviral Therapy**
            - HIV-associated Thrombocytopenia
    - Von Willebrand Disease
        - **DDVAP**
            - Releases vWF from stores
        - **Humate-P**
            - Factor VIII concentrate rich in vWF
        - **Cryoprecipitate**
            - GOLD STANDARD
            - Prepared from plasma and contains fibrinogen, vWF, factor VIII, factor XIII, and fibronectin
    - Hemophilia
        - No procedures without replacement therapy (avoid them!)
        - Hemophilia A
            - **Factor VIII**
                - Mild Hemorrhage: 15 u/kg
                - Major Hemorrhage: 25 u/kg
                - Life-threatening Lesion/Surgery: 40-50 u/kg
        - Hemophilia B
            - **Factor IX** (double values for Hemophilia A)
                - Mild Hemorrhage: 30 u/kg
                - Major Hemorrhage: 50 u/kg
                - Life-threatening Lesion/Surgery: 80 u/kg
        - **E-Aminocaproic Acid**
            - Useful adjuvant
    - Factor XI Deficiency
        - **Fresh Frozen Plasma**
            - Long ½ life, so single dose enough
        - **E-Aminocaproic Acid**
            - Adjuvant
    - Factor XII and Above
        - No treatment needed



- Anticoagulation Therapy
    - *REMEMBER: Cannot inhibit clot formation without increased risk of hemorrhage!*
        - *Hemorrhage: consequence of overaggressive therapy*
        - *Thrombosis: consequence of underagressive therapy*
    - Types of Antithrombotics
        - Antiplatelet Agents
            - For **ARTERIAL** thromboembolic disease
        - Anticoagulants
            - For **VENOUS** thromboembolic disease
        - Thrombolytic Agents

- Dissolution of thrombi

- Antiplatelet Therapy (arterial)
  - Irreversible COX inhibitor
    - **Aspirin**
      - Blocks 1º (not 2º) wave of platelet aggregation
      - **Low Dose**: inhibits TxA2 production→ blocks both platelet aggregation *and* vasoconstriction
      - High Dose: inhibits both TxA2 and Prostacycline (PGI2)→ PGI2 inhibits platelets and vasodilates, so high dose therapy not as dramatic
  - Competitive COX inhibitor
    - **NASAIDs**
      - Blocks 1º and 2º wave of platelet aggregation
      - As such, inhibit clot formation to greater extent than aspirin
      - Blocks aspirin effect on platelet, thus aspirin should be taken BEFORE you take NSAID
  - **Dipyridamole**
    - Enhances aspirin's effect on platelet aggregation; aspirin adjuvant!
  - GP IIb/IIIa inhibitors
    - Most potent antiplatelet medications→ render platelet count to functional zero
    - Block fibrinogen-induced platelet aggregation
    - Use: to block immediate restenosis following PCI
    - Too potent for long-term use
    - Only IV
    - Examples: **abciximab**, **eptifibatide**, **tirofiban**
  - ADP Receptor Inhibitors
    - Block $P2Y_{12}$ receptor, inhibiting ADP-induced activation of platelets
    - Use: unstable angina; MI (both NonSTEMI and STEMI), PCI, peripheral vascular disease, stroke
    - Side effects: TTP and neutropenia (ticlopidine)
    - Warning: PPIs can block drug activity
    - All long-acting; no reversing agent
    - Examples: **clopidogrel, prasugrel**, ticlopidine (rare)
- Anticoagulant Therapy (venous)
  - Inhibitors of coagulation cascade
  - All agents prevent propagation of clot; none dissolve already formed clots
  - Risk factors for venous thromboembolic disease:
    - Major surgery (leg>pelvic>abdominal/thoracic)
    - Acute MI
    - Trauma
    - Paralytic stroke

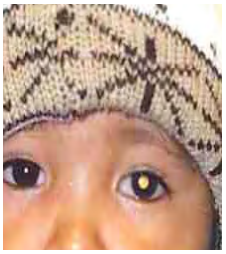
        - Cancer
        - Spinal cord injury
        - Pelvic fracture
- Heparin/Heparin Derivatives
    - Potentiate antithrombin III's inactivation of active enzymes of clothing cascade (IX, X, XI, thrombin)
    - Useful in preventing *and* treating thromboembolic dz
    - Unfractionated Heparin
        - Advantages: cheap; fully reversible with *protamine;* short ½ life
        - Disadvantages: IV infusion, variable bioavailability; monitoring of INR required; higher risk of complications (HIT)
        - Example: **heparin**
    - Low Molecular Weight Heparins
        - Advantages: higher bioavailability; no monitoring required; long ½ life; much lower risk of complications (HIT)
        - Disadvantages: expensive; longer-acting, not as reversible; renal excretion; *black-box warning for use with regional anesthesia*
        - Examples: **enoxaparin** (Lovenox®), **dalteparin** (Fragmin®), **tinzaparin** (Innohep®)
- Factor Xa inhibitors
    - Active moiety of heparin, except only inhibits Factor Xa
    - Bioavailability is 100%, can be given 1xday
    - SE: *incidence of wound hematomas*
    - Do not see black-box warning against use with regional anesthesia as seen with LMWH
    - Example: **fondaparinux** (Arixtra®)
- Direct Thrombin Inhibitors
    - Block active site of thrombin, both free and clot-bound
    - More potent than heparin
    - **Lepirudin** (Refludan®)
        - Problematic in renal disease
        - Not reversible
        - Approved for HIT
    - **Argatroban®**
        - Problematic in liver disease
        - Not reversible
        - Approvide for HIT and ACS
    - **Bivalirudin** (Angiomax®)
        - Not reversible
        - Approved for unstable angina, angioplasty
    - **Desirudin**
        - Approved for DVT prophylaxis in HIT

- **Dabigatran (NEW)**
  - Oral
  - Approved for VTE prophylaxis (soon possibly for stroke prophylaxis in pts with A-fib)
  - Touted as Warfarin replacement
- **Coumadin** (Warfarin)
  - Inhibits Vitamin K dependent carboxylase activity
  - Does not affect already synthesized proteins
  - Monitor using PT time
  - Antidote: Vitamin K

o Thrombolytic Therapy
- Lyses already formed clots: coronary thrombi in M.I.
- Doesn't discriminate b/w therapeutic and pathologic thrombi
- Markedly increased risk of hemorrhage compared with other antithrombotic therapies
- **Streptokinase**
  - Increases plasminogen-plasmin complex activity
- **Urokinase**
  - Activates plasminogen directly
- **Tissue Plasminogen Activator (TPA)**
  - Made by endothelial cells
  - Increased affinity for fibrin-bound plasminogen
  - Activates plasminogen directly

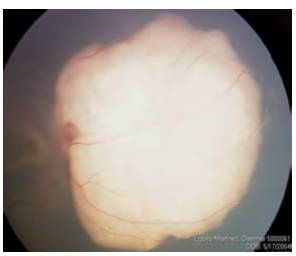
Lecture #8: 11/19- Pediatric Cancers

- Pediatric Cancer Epidemiology
  - 1 in 750 20-yo alive today in the U.S. is a survivor of childhood cancer
  - Overall survival for childhood cancer is ~80%
  - **Leukemia** is most common childhood cancer
  - **Brain tumors** comprise 2nd most common childhood cancer
  - Most childhood cancers arise **before age 5**
- **Retinoblastoma**

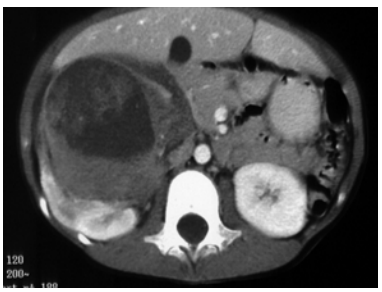


  - Primitive neuroectodermal tumor of the **retina**
  - One or both eyes can be affected
  - 3 forms: *familial, sporadic heritable, sporadic non-heritable*
  - Paradigm for **two-hit** model of carcinogenesis
  - Genetics
    - Gene: ***RB1*** (Chr. 13q)
    - Encodes pRb, nuclear phosphoprotein that halts cell cycle by exerting negative regulatory effect on gene expression
    - Mutations can be germline (bilateral) or somatic (unilateral)
  - Familial Retinoblastoma
    - **Inherited germline RB1 mutation**
    - 10% of RB cases
    - ~95% with familial RB1 mutation develop RB
      - Mostly **bilateral**

Large intraocular tumor

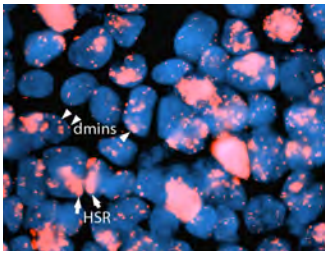


        - Every cell in body contains 1st hit
        - **2nd hit occurs in retinal cell post-conception**
        - Lifelong risk of secondary malignancy (~30%)
    - Sporadic Heritable Retinoblastoma
        - **De novo germline RB1 mutation** (i.e. not in parents)
        - 30% of RB cases
        - **Same risk of developing RB (95%) as in familial RB**
            - Mostly **bilateral**
        - Able to pass mutation to offspring
        - Same risk of secondary malignancy as familial RB (~30%)
    - Non-Heritable Retinoblastoma
        - **Acquire two distinct somatic *RB1* mutations in single cell post-conception**
        - 60% of RB cases
        - Develop ONLY **unilateral** disease
    - Signs and Symptoms
        - **Leukocoria** (lack of red reflex in photographs, see *fig*)
        - Often not visible until tumor large enough to affect vision
        - Usually painless
        - Signs of aggressive dz: proptosis, erythema, pain
    - Treatment
        - Unilateral RB: **enucleation** of affected eye
        - Bilateral RB: **local therapy** of less affected eye to preserve vision
- **Wilms Tumor**
    - **Renal** tumor of childhood
    - Associated congenital anomalies:
        - **Aniridia**: absense of an iris
        - **Hemihypertrophy**: one side of body larger than other
        - **Cryptochodrism**: undescended testicle
        - **Hypospadias**: abnormal male urethral position
        - Non-Overgrowth Syndromes
            - Wilms Tumor-Aniridia-GU Anomaly- Retardation Syndrome (**WAGR**)
        - Overgrowth Syndrome
            - Beckwith-Widemann Syndrome
    - Genetics
        - ***WT1***: Wilms tumor suppressor gene (Chr. 11p13)
        - Encodes zinc finger transcription factor
        - Somatic mutations of *WT1* found in only 5-20% of sporadic WT
        - Wilms Tumor predisposing syndromes:
            - **Wilms Tumor-Aniridia-GU Anomaly- Retardation Syndrome (WAGR)**
                - Large deletion at Chr. 11p13
                    - Deleted Genes
                        - WT1: GU anomalies

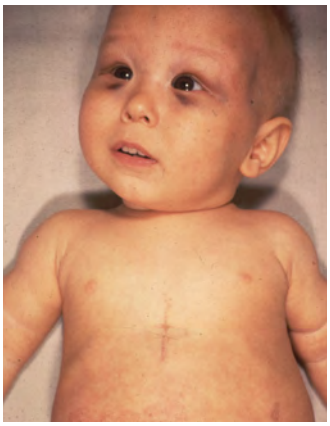


        - PAX6: aniridia
    - **>50% risk of developing Wilms tumor**
- **<u>Beckwith-Wiedemann Syndrome</u>**
    - Overgrowth syndrome (hemihypertrophy)
    - Imprinting disorder arising from mutation in genes from imprinted cluster at Chr. 11p15.5
    - **5-7% develop Wilms tumor**
- Pathology
    - <u>Classic nephroblastoma</u>: blastemal, stromal, epithelial cells
    - Favorable vs. anaplastic histology
        - Anaplstic have high incidence of p53 mutations
- Signs and Symptoms
    - Abdominal mass (60%), HTN (25%), hematuria (15%)
- Treatment
    - **Radical Nephrectomy**: standard of care
    - **Dactinomyin**: chemotherapeutic adjuvant

- **Neuroblastoma**
    - Most common cancer of infancy (2x rate of leukemia)
    - Originate from **neural crest cells** that normally give rise to the adrenal gland and sympathetic ganglia
    - Neuroblastomas <u>can spontaneously regress</u> after prenatal diagnosis via ultrasound
    - Genetics
        - Increased DNA index (hyperdiploid) in 67%
        - **Deletions in Chr. 1p**
            - Indicates **tumor suppressor gene** resides at this locus
        - ***MYCN* amplification**
            - The more advanced the stage of tumor at diagnosis, the more likely the presence of MYCN amplification, and the poorer the prognosis
            - MYCN amplification can be stained with FISH (*fig*.)
                - *Double minutes* & *homogenous staining regions*

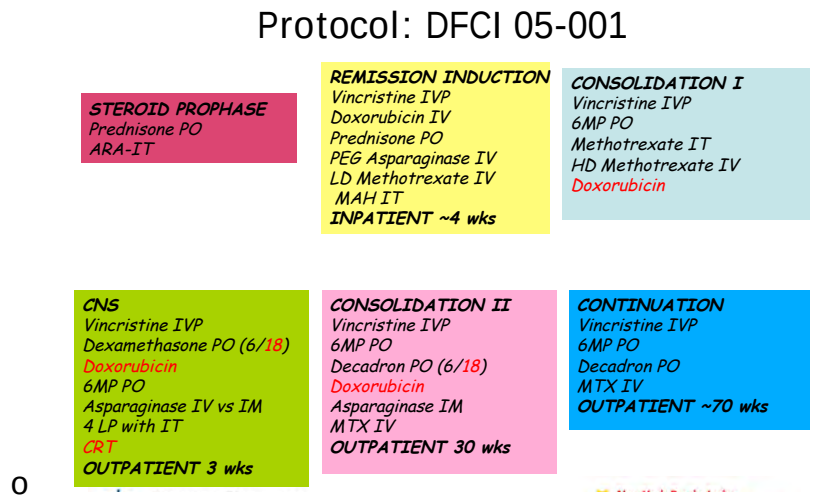


    - NB can originate from any site in sympathetic nervous system
        - Primary tumor: adrenal gland (>1yo), cervical/thoracic (<1yo)
        - Metastatic: bone marrow (70%), bone (50%), etc.
    - Signs and Symptoms
        - Metastatic NB (see *fig*)
            - **Proptosis**
            - **Periorbital echymoses**
            - **Bone Pain** (irritability, limp, refusal to walk)
        - Opsoclonus-Myoclonus Syndrome
            - "Dancing eyes and dancing feet"
            - Acute cerebellar encephalopathy, truncal ataxia, random rapid eye movements
            - Therapy: ACTH, IVIG, chemotherapy



    - Diagnosis/Work-Up

        - Urinary catecholamine metabolites, homovanillic acid (HVA) and vanillymandelic acid (VMA)
        - Eval. of 1º tumor: CT Scan/MRI
        - Eval. of metastatic disease: bone scan, bone marrow asiprates
- **Childhood Acute Lymphoblastic Leukemia (ALL)**
    - Pathophysiology
        - Single lymphocyte loses ability to differentiate and undergo apoptosis, and multiplies abnormally
        - Leads to **accumulation of abnormal lymphocytes (blasts) in bone marrow** and **crowding out** of normal marrow elements
        - Results in varying degrees of **anemia**, **thrombocytopenia**, **granulocytopenia**
    - Epidemiology
        - Boys>Girls
        - Caucasians
        - No consistent or common etiologies
    - Signs and Symptoms
        - **Fatigue** (2º to **anemia**), **bleeding** (2º to **thrombocytopenia**), lymphadenopathy, bone pain, hepatosplenomegaly, fever
    - Diagnosis
        - CBC, blood smear, CXR, LP for CSF infiltration, etc.
        - **Bone Marrow Aspirate and Biopsy** (diagnotic test)
        - Refining Diagnosis
            - Flow Cytometry (Immunophenotyping)
                - As cells mature, different surface antigens exp.
                - Can assess stage of cell by identifying antigens
            - Cytogenetics
                - Hyperdiploidy (good), **hypodiploidy (bad)**, haploid (very bad)
                - Chromosomal abnormalities
                    - **Philadelphia Ch.**, t(9,22): **poor** prognosis
                    - MLL rearrangements: **poor** prognosis
                    - TEL/AML, t(12,21): good prognosis
    - Treatment
        - Complicated:



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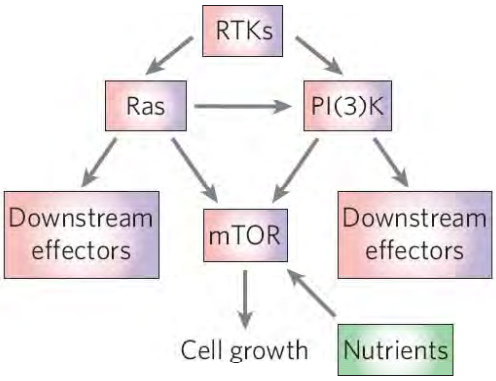
Lecture #9: 11/22- Carcinogenesis

- 1/3 to 1/4 of people will develop cancer before they die
- Gradual rise in prostate cancer incidence over the past century because of increased screening and detection of previously undetected cancer
- Colon and rectal cancer incidence is decreasing, probably due to awareness
- Mortality from cancer is going down, even though incidence is going up
    - Good detection and treatment
- How do you get cancer?
    - **Smoking**
        - Causes many cancers
            - **Lung**, pancreatic, esophageal, head/neck, **bladder**
    - Diet
        - Japan: westernization of diet associated with increased colon cancer (instead of gastric cancer) and increased breast cancer
        - US: red meats contain carcinogens and cause colon cancer
    - Fumes
        - Benzene→ leukemia
    - Infections
        - Human Papillomavirus (HPV)
            - Immortalize cells by inactivating tumor suppressors
            - Transmitted via direct contact
        - HBV, HCV
            - Hepatocellular carcinoma (HCC)
        - EBV
            - Burkitt's Lymphoma (Africa)
                - Immortalizes B-cells
                - Infection earlier in life in Africa, which confers risk for lymphoma vs. mono in western society
        - HTLV-I, II
            - Acute T-cell Leukemia
        - *H. pylori*

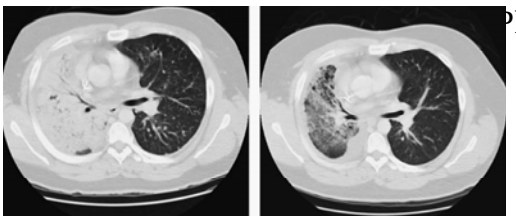
            - Gastric cancer
    - Power Lines
        - **DO NOT** cause cancer
        - Wavelength of electromagnetic radiation generated by power lines is too large to cause DNA damage
- Cancer Causation
    - For proof of causation, need:
        - Mutation potential, epidemiologic evidence, and animal model
    - Example
        - **Asbestos**
            - **Crocidolite fibers** from asbestos disrupt mitotic spindle and cause mutations in cells
            - Inject crocidolite fibers into rat and cause mesothelioma
- Mutagenesis in Cancer
    - Spontaneous mutations: 15%
    - Hereditary mutations: 5%
    - **Exposure to mutagens/genetic predisposition to mutations**: 80%
- Clonal Origin of Cancer
    - All cancer starts in a single cell
    - Mutation results in clonal cell growth
    - In situ→ metastatic
    - Development of additional mutations
- Metastasis
    - Required intravasation and subsequent extravasation of vessel
- Cancer Genes
    - **Tumor suppressors**: <u>recessive</u>→ must loose 2 alleles to cause cancer
    - **Oncogenes**: <u>dominant</u>→ single allele mutation results in cancer
- Prevention of Cancer
    - Decrease exposure to carcinogens
        - Taxation of cigarettes, public awareness campaigns, etc.
    - Diet
        - Decreased fat, salt, smoked foods; increased freshness
    - Decrease exposure to hormones
        - Endogenous/exogenous estrogen causes cancer
        - Pregnancy = progesterone, which checks estrogen's carcinogenic affect
    - Cancer Preventive Agents
        - ASA/COX→ prevent gastric cancer (but inc. CAD)

Lecture #10: 11/22- Signal Transduction

- Protein Kinase:
    - Phosphorylates a protein (and, in many cases, activates it)
- Protein Phophatase:
    - Dephosphorylates a protein (and, in many cases, deactivates it)
- Signaling Kinases
    - **Cell surface** and **intracellular** kinases



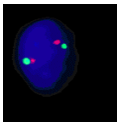
- Oncogenes
  - Dominant mutations: just 1 abnormal allele results in cancer activity)
  - Hierarchical activation: pathway activation in *cascade* form
  - Drug resistant via reactivation of blocked pathway
- **Receptor Tyrosine Kinase (RTK)**
  - Families
    - **Epidermal growth factor (EGFR)**, insulin-like growth factor (IGFR), vascular endothelial growth factor (VEGFR), fibroblast growth factor (FGFR), **human epidermal growth factor 2 (HER2)**
  - **Epidermal Growth Factor Receptor (EGFR)**
    - Physiology
      - Binding of EGF results in dimerization of 2 adjacent EGFRs, and subsequent **<u>auto</u>transphosphorylation**
      - EGFR phosphorylation reveals docking site for **downstream effectors→** cascade!
      - Result is increased activity of transcription factors with various functions
    - Oncogenesis
      - **Mutation** in <u>kinase domain</u> of EGFR results in **constitutively active kinase activity**

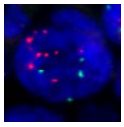


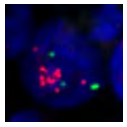
Pharmacology

- **<u>Erlotinib</u> (Rx Lung Cancer)**
  - Mechanism of Action
    - Fits into active site of EFGR, blocking ATP binding site, and thus preventing EGFR autophosphorylation
    - Dramatic effect in certain **lung cancers**!
  - Predictors of Benefit
    - Lung tumors displaying **EFGR mutation predict major clinical benefit**
    - Conversely, tumors displaying **additional HER2 mutations or wildtype EGFR predict poorer/no clinical benefit**
  - Problem

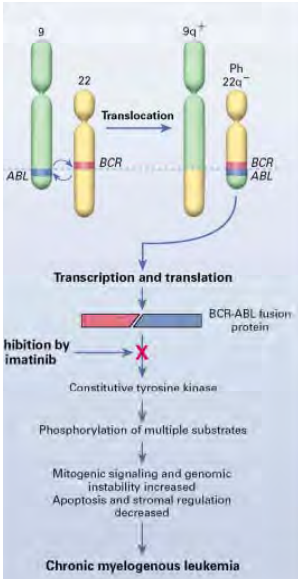
        - **Resistance**: tumors develop new mutations (T790) in erlotinin binding domain, blocking drug's binding; various other resistance mechanisms
    - **Human Epidermal Growth Factor Receptor 2 (HER2/neu)**
        - Oncogenesis
            - Amplified or over-expressed in 25% of **breast cancer**
            - Amplification detected with FISH (see *fig*)


Normal


Abnormal low amplification


Abnormal high amplification

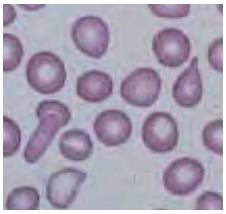
        - Pharmacology
            - **Trastazumab**: sensitizer of taxanes (anti-mitogens); increases response in HER2 amplification tumors
            - **Doxorubicin**: indicated in HER2 amplification tumors
- **RAS**
    - $RAS\text{-}GDP_{INACTIVE} \rightarrow (GEF) \rightarrow \underline{RAS\text{-}GTP_{ACTIVE}} \rightarrow (GAP) \rightarrow RAS\text{-}GDP_{INACTIVE}$
    - RAS Mutations in Cancer
        - K-RAS: lung, colon, pancreas, endometrium, biliary
        - Mutations affect **GTP-ase association site** on RAS, thus **GTP always bound**, and **RAS is constitutively active**
    - Prenylation
        - RAS undergoes post-translational modification, in which fatty acid are added to the protein, in a process called **farnesylation**
        - If block this process, can block transformation of cells by +RAS
- **BCR-ABL Fusion**

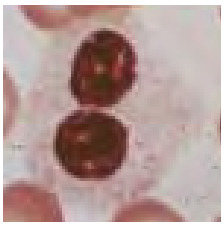


    - Seen in *Chronic Myelogenous Leukemia* (CML)
    - **Philadelphia Chromosome**
        - BCR-ABL fusion is the result of chromosomal translocation, t(9,22), in which BCR is brought directly upstream of ABL
        - Translocation results in loss of ABL's N-terminal cap, which usually autoinhibits the ABL's kinase activity; with BCR-ABL fusion, this autoinhibition is lost
    - Pharmacology
        - **IMATINIB (Gleevec®)**
            - Standard of care for Rx for CML, GISTs
            - MOA:
                - **Binds to ABL and inhibits kinase activity**
            - Problems
                - **Resistance**
                    - Alteration in imatinib binding pocket
                    - Results in CML *blast crisis*
        - **Dasatinib**
            - Helps overcome imatinib resistance
            - MOA:
                - Binds to ABL pocket in different manner, so rescues anti-ABL activity in imatinib resistance

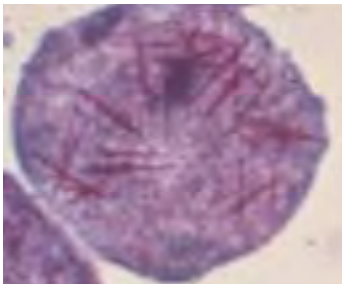
Lecture #11: 11/23- Acute Leukemia

- In acute leukemia, major cell type is an immature **precursor cell**, i.e. a **blast**
- Cells acquire mutation that **prevents differentiation**; cells not dividing!
- Genetic Damage
  - Majority have visible **chromosomal abnormality** (i.e. translocation)
    - But point mutations, rearrangements, and deletions also seen
  - DNA change can lead to:
    - **Conversion of proto-oncogene→oncogene**
      - Via **amplification** (ie. *myc*) or **alteration** (ie. BCR-ABL) of proto-oncogene product
    - Inactivation of tumor suppressor gene
      - Unchecked cell growth, regardless of DNA damage
  - Causes of DNA Damage in Leukemias
    - **Radiation**, **carcinogens** (*benzene*, chemotherapy), hereditary chromosomal or DNA-repair disorders, viruses
- Major Categories
  - **Myelodysplastic Syndrome (MDS)**



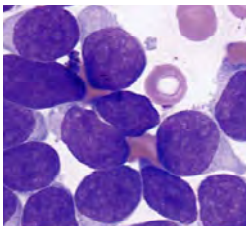


    - Usually elderly patients (50-70 yo)
    - Majority idiopathic; can be seen w/ hx of chemo/radio-therapy
    - Myeloid precursors **retain terminal differentiation capability**, but occurs in a disorders and **ineffective fashion**;
    - Results in:
      - **Variable cytopenias** (because not differentiating into all myeloid lineages)
        - Neutropenia and thrombocytopenia
      - Anisopoikilocytosis (RBCs of variable size & shape; *fig*)
      - Neutrophil hypogranularity and hyposegmentation (*fig*)
      - Hypolobulated megakaryocytes
    - Abnormal stem cell clone in bone marrow is unstable, leading to acquisition of additional mutations and **eventual transformation to acute myeloid leukemia (AML)**
  - **Acute Myelogenous Leukemia (AML)**

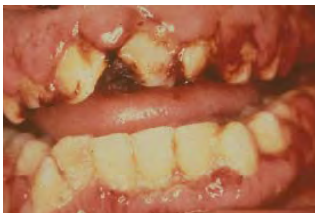


    - Bone marrow filled with **precursor myeloblast**
    - From Robbins:
      - *Neoplastic cells are blocked at some **early stage** of myeloid cell development; immature myeloid blasts (which can exhibit evidence of granulocytic, erythroid, monocytic, or megakaryocytic differentiation) **accumulate in the bone marrow**, replacing normal elements→**marrow failure**, and frequently circulate in the peripheral blood*
    - **DIC** common, as well as purpura and other signs of bleeding
    - Cells often contain multiple **Auer rods** (see *fig*).
    - Genetics
      - **t(15;17)** almost always present

        - Results in **PML-RARA gene fusion** which blocks promyelocyte differentiation & blocks apoptosis
    - Therapy
        - **Retinoic Acid**
            - Acts as **differentiating agent**!
            - Malignant clones **mature into neutrophils**, and live **normal neutrophil lifespan** before dying
            - **Relapse** possible because precursor stem cell in bone marrow is resistant to retinoic acid; but, if coupled with **chemotherapy**, progenitor cells in bone marrow die as well and excellent prognosis



- **Acute Lymphocytic Leukemia (ALL)**
    - Usually disease of **children** (as opposed to AML)
    - Arises in early progenitor B-cell or T-cell (B:T=4:1)
        - See Ig or T-cell receptor gene rearrangement
- Clinical Features of Acute Leukemias
    - **Marrow failure**
        - **Neutropenia→ infection** (opportunistic: i.e. aspergillus, etc.)
        - Anemia→ weakness, fatigue
        - Thrombocytopenia→ bleeding (**echymoses/purpura**)
    - **Hyperuricemia**
        - Tubular damage→ acute renal failure

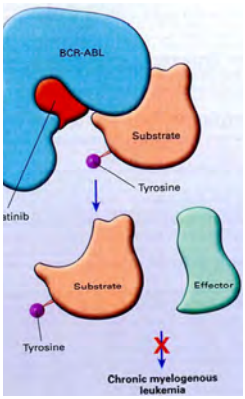


    - **DIC**
        - Thrombocytopenia/Abnormal clotting→ bleeding (intracranial hemorrhage)
    - Organ Infiltration
        - Bone pain, hepatosplenomegaly, **hypertrophied gums**, meningeal infiltration→ headaches
- Treatment of Acute Leukemias
    - Intensive **combination** therapy
    - Chemotherapy beyong remission
    - **CNS prophylaxis with ALL**
    - Bone marrow transplantation in select patients
    - Supportive therapy (allopurinol, transfusions, antimicrobials)

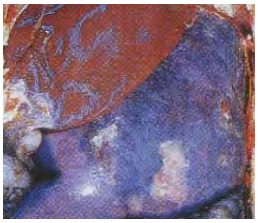
Lecture #12: 11/23- Myeloproliferative Disorders

- In myeloproliferative disorders, usual cell phenotype is **differentiated cell** of myeloid lineage
- There is an increase in one or more formed elements of the peripheral blood (seen on smear)
- From Robbins:
    - *Most, if not all, myeloproliferative disorders are associated with an abnormal **increase** in the **activity** of one or another **tyrosine kinase**, which appears to stimulate the same signaling pathways are normally activated by hematopoietic growth factors*
- **Chronic Myelogenous Leukemia (CML)**

- See increased pool of clonal precursors that are committed to becoming myeloid cells (i.e. *erythroid, megakaryocytic, and granulocytic* cell lines)→ most differentiate into **mature cells**
- Genetics
  - **Philadelphia Chromosome:** CML associated with t(9;22)
  - Product is BCR (22)-ABL(9) fusion gene/protein→*bcr-abl*
  - Leads to **constitutively active tyrosine kinase** (*abl* portion)
  - Results in <u>proliferative</u> signaling cascade, <u>decreased apoptosis</u>
- Signs and Symptoms
  - <u>Chronic Phase</u> (3-4 years duration)
    - **Splenomegaly**
    - Weakness, weight loss, purpura
    - Thrombocytosis
    - Anemia (normal MCV)
  - <u>Blastic Transformation/Blast Crisis</u> (after 3-4 years of CML)
    - *Resembles acute leukemia*
    - Weight loss, fever, sweats, bone pain
    - Worsening: splenomegaly, anemia, thrombocytopenia
    - Resistance to therapy
    - Death in weeks or months
- Blood/Marrow Analysis
  - <u>Chronic Phase</u>
    - WBC increased
    - Entire granulocytic spectrum (PMNs, basos, eos) on film
    - **Low *neutrophil alkaline phosphatase***
      - CML cells are abnormal and don't produce this
  - <u>Blastic Transformation/Blast Crisis</u>
    - Myelo**blasts** & promyelocytes +++ (precursor cells)
    - Philadelphia chromosome with additional mutations
- Treatment
  - Hydroxyurea
    - Inhibits ribonucleotide reductase→ dec. DNA synthesis
    - Reduces blood counts, but does not improve outcomes
  - Interferon-alpha
    - 10-15% become Philadelphia chromosome negative
  - **<u>Imatinib</u> (Gleevec®)**
    - Tyrosine kinase inhibitor; **targets *abl*** (see *fig*)
    - Potent, low toxicity
    - 60% becomes Philadelphia chromosome negative
  - **Allogeneic Bone Marrow Transplantation**
    - Potentially curative
    - From normal donor
    - See ***graft-vs-malignancy***, where graft T-cells kill cancer
    - Complications: 10-25% mortality from *graft-vs-host dz.*



- **Myelofibrosis with Myeloid Metaplasia (MMM)**
  - **Bone marrow fibrosis**

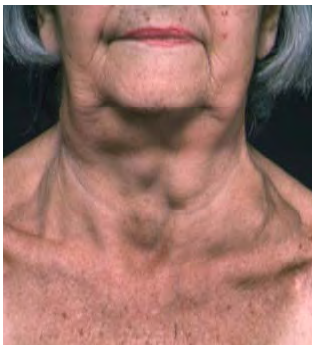


- Fibrosis often follows period of **elevated WBCs and platelets**
- Because of bone marrow failure, **extramedullary hematopoiesis** (i.e. outside of bone marrow) ensues, particularly in the **spleen** (and liver)→ **splenomegaly** (with splenic infarcts; *fig*), hepatomegaly
- However, extramedullary hematopoeisis is not sufficient→ can result in **severe anemia and thrombocytopenia**
- Associated with JAK2 mutations

- **Polycythemia Vera (PV)**
  - Abnormal cone of hematopoietic stem cells are increasingly sensitive to growth factors
  - Results in **elevation of all cell counts**: RBCs, WBCs, platelets
  - Hallmark: **very elevated RBC mass (+++hematocrit)**
  - Associated with JAK2 mutations
- **Essential Thrombocythemia (ET)**
  - Similar to polycythemia vera, but specific to **megakaryocytes**
  - See increased **platelets**
  - Least aggressive of the myeloproliferative disorders
  - Associated with JAK2 mutations

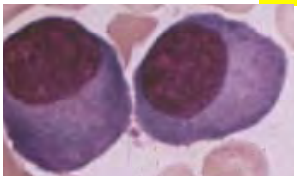
Lecture #13: 11/24- CLL and Myeloma

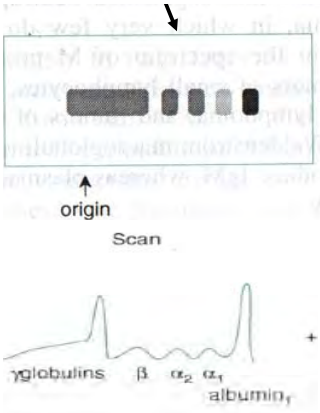
- Pre-B-cell
  - Malignancy: acute lymphocytic leukemia (ALL)
- B-Cell
  - Malignancy: **chronic lymphocytic leukemia (CLL)**
  - Decreased gamma globulin
- Transformed B- Cell
  - Malignancy: macroglobulinemia
  - Monoclonal IgM
- Plasma Cell
  - Terminally differentiated B-cell
  - Malignancy: **myeloma**
  - Monoclonal Ig or light chain
- **Chronic Lymphocytic Leukemia**
  - Most common leukemia
  - Usually age >50 yo
  - Increased **proliferation** and **accumulation** of neoplastic, immunologically incompetent, clonal **lymphocytes (B-cell >99%)**
  - Signs and Symptoms
    - Highly variable
    - **Asymptomatic** or vague, nonspecific symptoms
    - Recurrent infection
    - **Lymphadenopathy** (60%; see *fig*), splenomegaly (50%), hepatomegaly (<40%)
  - Diagnosis
    - Lymphocytosis in blood and bone marrow



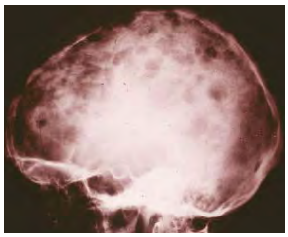
        - **B-cell monoclonality** (mutation in B-cell committed to either kappa or lamda chain immunoglobulin)
        - **Hypoimmunoglobulinemia→** inc. risk of bacterial infection
        - Autoantibodies: immune thrombocytopenia (bleeding)
    - Prognosis
        - Mean survival: 50-60 months
        - Range: few months to >20 years
    - Treatment
        - Asymptomatic: no therapy required
        - Symptomatic:
            - Radiation (locally)
            - Chemotherapy: fludarabine, alkylators (and combo)
            - **Monoclonal antibodies: rituximab, campath**
            - Stem cell transplantation: only in young pts.

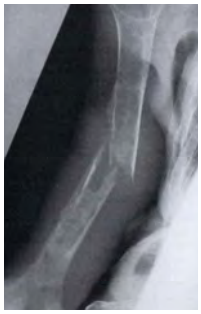
- **Multiple Myeloma**





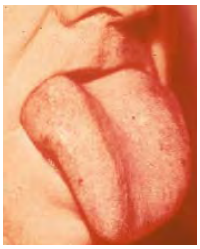
    - Malignancy of **plasma cells** (terminally differentiated B-cells)
    - Blacks>whites (2x)
    - Age range: 20-100 yo (peak: 70 yo)
    - Cause: unknown
    - Cardinal Features
        - **Plasmacytosis in bone marrow** (see *fig*)
            - Plasma cells present in **sheets**
            - Can be multinucleated (dysplastic)
            - Can see **Ig inclusions**
            - Comprise >10% of marrow cells (or much higher)
        - Increased **monoclonal immunoglobulin** in **serum/urine**
        - **Lytic bone disease**
            - Unbalanced **osteoclast** activity
                - Via secretion of *RANKL*
            - Osteoporosis
            - Axial skeleton involved
            - Hypercalcemia/-uria





    - Diagnosis
        - Serum monoclonal immunoglobulin (75-80%)
            - See gamma "**spike"** on electrophoresis (see *fig*)
        - Confirmed with **immunofixation electrophoresis**
    - Presentation
        - Early: asymptomatic, incidental diagnosis
            - Monoclonal protein on electrophoresis
            - Mild marrow plasmacytosis
            - Solitary plasmacytoma (10%)
        - Late: symptomatic (majority)
            - Bone pain (usually lower back)
            - Pneumococcal infection
            - Systemic symptoms (e.g. weakness, weight loss)
        - **Hyperviscosity Syndrome**

- Due to aggregating monoclonal protein
- Circulatory insufficiency, abnormal hemostasis
- Manifestations: bleeding, dyspnea, encephalopathy, **visual disturbances** (exudates, retinal vein sausaging)

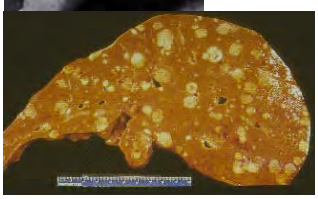


- Amyloidosis
  - Due to light chain deposition in tissues
  - Lamda > kappa amyloid
  - Organs commonly involved: skin, tongue (glossomegaly, *fig*), heart, peripheral nerves, kidneys, soft tissues (hypertrophy)
  - Therapy: stem cell transplantation

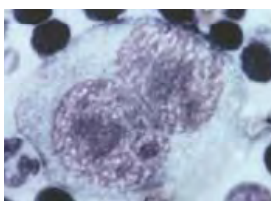
- ***Benign Monoclonal Gammopathy***
  - *Another cause of monoclonal immunoglobulin*
  - *Monoclonal Ig as isolated finding → normal, benign disease*
  - *No bone disease, anemia, or renal dysfuncton*
  - *About 10% eventually develop classical multiple myeloma*
- Therapy
  - Bisphosphonates (for osteoporosis)
  - Radiotherapy
  - Corticosteroids
  - Chemotherapy (anthracyclines and alkylators)
  - Thalidomide (blocks blood vessel growth)
  - Bortezomib (proteasome inhibitor)
  - Stem cell transplantation

Lecture #14: 11/24- Lymphoma

- Diagnosis of lymphoma:
  - **Lymph node biopsy**
    - Cell: morphology, cytochemistry, cytogenetics, molecular
- Lymphocyte Characteristics (B-cells and T-cells)
  - Origin: marrow (B), thymus (T)
  - Immune function: humoral (B), cellular (T)
  - Surface/Cytoplasmic Ig: ++ (B), - - (T)
  - T-Cell receptor: - - (B), ++ (T)
  - Immunostaining: CD19,20+ (B), CD3,4,8+ (T)
  - Gene rearrangements: Ig (B), TCR (T)

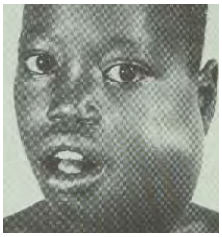


- **Hodgkin's Lymphoma**
  - Bimodal age distribution (20-30 yrs and >50 yrs)
  - Genetic and environmental pathogenesis
  - Lymph node involvement, with RARE extranodal involvement
  - Clinical
    - **Lymphadenopathy**: cervical> mediastinal> paraaortic
    - Commonly unilateral
    - Paraaortic nodes: tracheal deviation/collapse (→stridor)
    - Spleen commonly filled with lymphoma (*fig*)
  - Lymph Node

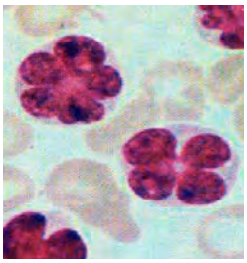


        - Effacement of normal lymphoid follicles
        - **Reed-Sternberg Cells** (see *fig*)
            - Large, bi-nucleated cell (owl eyes)
    - PET Scan
        - Used to follow/stage lymphoma following diagnosis
    - Therapy
        - **Combination Chemotherapy + radiation**: cure rates in 90%
            - MOPP
                - **M**ustargen, **O**ncovin (vincristine), **P**rocarbazine, **P**rednisone
        - In resistant lymphomas:
            - **Autologous Stem Cell Transplantation**
    - Outcomes
        - Patients tend to **die from other causes related to early treatment** of Hodgkin's lymphoma
            - Fatal: myelodysplastic disease, acute leukemia, non-Hodgkin's lymphoma, solid tumors, sepsis
            - Serious: myocardial damage (from doxorubicin), lung fibrosis (bleomycin), sterility, growth abnormalities, infection
- **Non-Hodgkin's Lymphoma (NHL)**
    - Distinguishing features from Hodgkin's Lymphoma
        - **Absence** of Reed-Sternberg cells
        - **Middle-aged** incidence
        - **Extra-nodal** sites common
    - Dozens of forms of non-Hodgkin's Lymphomas
        - **Indolent** (follicular architecture retained)
        - **Aggressive** (diffuse, destructive architecture)
    - Indolent Form
        - **Follicular NHL**
            - Most common indolent NHL
            - t(14;18) in 85%→ IgH/BCL2 fusion→ **over-expression of anti-apoptotic BCL2**
            - 33% transform to aggressive NHL
            - Very treatable, though incurable
            - Treatment:
                - Alkylators
                - **Rituximab** (anti-CD20) + chemotherapy
                    - Humanized antibody
                    - Action: 1) pro-apoptosis, 2) complement or 3) antibody dependent cytotoxicity
    - Aggressive Form
        - **CNS** commonly involved
        - Paradoxically: **more curable than indolent NHL** (i.e. 30-60% cure rate), because cells more metabolically active so more vulnerable to effects of cytotoxic therapy

- Treatment
  - Early stage disease:
    - **RCHOP x 3 cycles + radiation**
      - **R**ituximab**, C**yclophosphamide, **H**ydroxydaunorubicin (doxorubicin), **O**ncovin (vincristine), **P**rednisone
  - Late stage disease:
    - RCHOP x 6 cycles +/- radiation
  - Progressive or relapsed disease
    - Autologous stem cell transplant



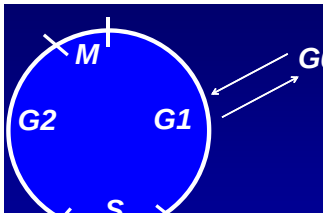
- **Burkitt's Lymphoma**
  - High-grade, B-cell malignancy
  - Seen in **Africa**
  - Causes
    - 100% associated with **EBV infection** (virus immortalizes B's)
    - Sporadic: AIDS
  - Genetics
    - Almost all, whether EBV or sporadic, will have **t(8;14)**→ *myc* to Ig promoter region→ cellular proliferation
  - Treatment
    - Cure rate ~ 90% with combination therapy (although regimen is highly toxic)
- **Cutaneous T-Cell Lymphoma**
  - Early: associated with **cutaneous lesions**
    - Pruritus, eczematous eruptions, plaques, erythroderma, leonine faces, etc.
  - Late: eventually will involve lymph nodes and spleen→ weight loss, constitutive symptoms→ poor prognosis
    - Secondary infections
  - T-helper cell malignancy (CD4+)
    - Skin: lymphocytes
    - Blood: large lymphocytes w/ convoluted nuclei (**Sezary Cells**)



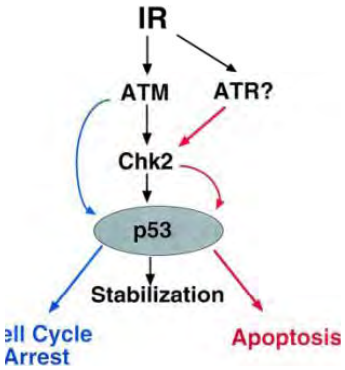
- **Adult T-Cell Leukemia**
  - Seen in **Japan**, Caribbean populations
  - **Lytic bone changes** similar to those in multiple myeloma
  - Blood smear: **flower cells** (see *fig*)
  - Results from infection with **HTLV1 infection**
  - Genetics
    - Clonal rearrangement of T-cell Receptor (TCR)
    - IL2 receptor over-expression

Lecture #15: 11/29- Cell Cycle Regulation

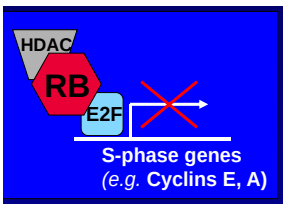
- From Dr. Garrett: "*You should understand the cell cycle, and the concept that factors can stimulate or inhibit cellular proliferation. Since the* ***retinoblastoma*** *and* ***p53*** *genes have come in multiple lectures, you should learn about those* ***genes****, their* ***gene products*** *and* ***role in carcinogenesis****.*

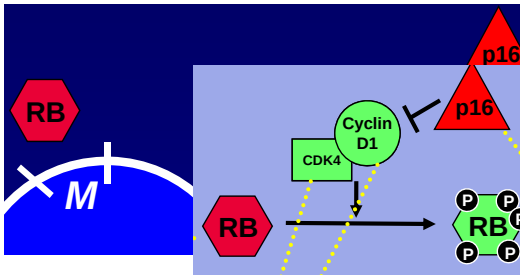


- **Cell Cycle**
  - Cell can be in resting phase: G0
  - Factors stimulate cell to enter active phase of cell cycles
  - **Deregulation of cell cycle is required** for tumorigenesis and/or progression of cancer
  - Mitogens: factors that stimulate entry into cell cycle
    - Growth factors, steroids, cytokines
    - ***Oncogenes***, cancer with gain of function
  - Antimitogens: factors that prevent entry in cell cycle
    - DNA damage, TGF-beta, hypoxia
    - ***Tumor suppressor genes*****,** cancer with loss of function
  - **Checkpoints**
    - Cell cycle **checkpoints** are in place so that the presence of DNA damage can be assessed; when (1) **DNA damage** is found, cell cycle (2) **arrest** occurs, allowing for either (3a) **repair** or cell (3b) **senescence**
    - DNA Damage and Cell Cycle Checkpoint Response
      - Detection of DNA damage→ activation of **ATM** (*ataxia telangiectasia*)
        - (A) activation of **p53** (*see below*)→ inactivation of CDK/cyclins→ cell cycle arrest
        - (B) activation of **Chk1/Chk2**→ cell cycle arrest



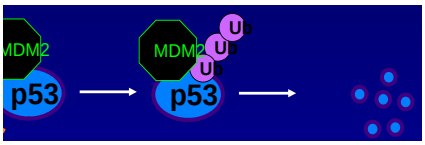
- **Retinoblastoma Protein, *RB***
  - RB is a **tumor suppressor** gene; the RB protein **inhibits proliferation** through formation of transcriptional repressor complexes that prevent transcription of S-phase (synthesis) genes
  - **CDK inhibitors (CDKi's)** are antimitogenic signals that block proliferative CDK signal, which themselves are dependent on cylclins
    - **CyclinD1-CDK4 phosphorylates RB and *inactivates* it**, blocking its anti-proliferative signal
    - **p16 = CDK4 inhibitor**, thus potentiating RB activity
  - Role in Cancer
    - Many cancers have either:
      - 1) **Loss** of **RB** activity
        - - - RB: retinoblastoma
      - 2) **Loss** of **p16/p27** activity
        - - - p27 (degraded by Skp2): **prostate** cancer
      - 3) **Amplification** of **CyclinD1/CDK4** activity
        - ++ CyclinD1: **breast** cancer





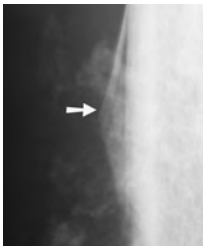
- **p53**
  - p53 is a **tumor suppressor** gene
  - p53 is activated when **DNA damage** is present
  - p53 activity can result in either:
    - **Apoptosis** (if DNA damage is severe), *or*
    - **Cell cycle arrest/senescence** (allows for DNA repair)

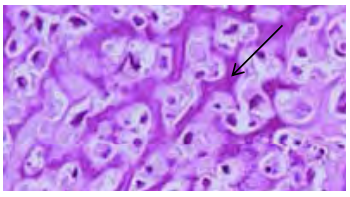
- Loss of p53 checkpoint activity can lead to continued proliferation, inefficient repair, secondary damage
- **<u>p53 Inactivation in Tumors</u>**
  - Mutation
    - <u>Somatic</u>: most commonly mutated tumor suppressor
    - <u>Inherited</u>: ***Li-Fraumeni Syndrome***
  - Deletion
    - Complete loss of p53 gene
  - Degradation
    - p53 protein can be degraded via **MDM2** <u>ubiquitination</u> (*targets p53 for proteasomal degradaton*)



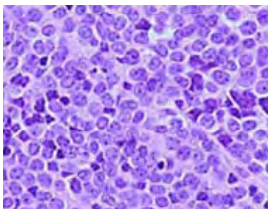
Lecture #16: 11/29- Sarcomas and Sarcoma Pathology

- Whereas "carcinoma" describes cancers of epitheliod morphology, "sarcoma" describes cancers of <u>fibrous</u> morphology
- **<u>Sarcoma</u>**
  - Represent less than 1% of all cancers
  - Tumors that arise from **mesenchymal** cells—the same cells that lead to formation of connective tissues such as *bone, muscle, fat, cartilage, tendon, ligaments, etc*.
  - Simply: **a cancer of the bone, cartilage, fat, muscle, blood vessels, or connective/supportive tissue**
  - Differentiated Types
    - Smooth muscle→ leiomyosarcoma
    - Skeletal muscle→ rhabdomyosarcoma
    - Fibrous tissue→ fibrosarcoma
    - Bone→ osteosarcoma
    - Cartilage→ chondrosarcoma
    - Fat→ liposarcoma
- **Pediatric Sarcomas**
  - ~10% of all pediatric cancers
  - **<u>Rhabdomyosarcoma</u>**
    - <u>Most common pediatric sarcoma</u>
    - Skeletal muscle origin, characterized by **myoblasts**
    - Embryonal (better prognosis) vs. alveolar (poorer prognosis)
    - Alveolar RMS→ PAX3-FKHR gene fusion
  - **<u>Osteosarcoma</u>**
    - <u>High-grade</u> tumor compose of fibroblasts, myofibroblasts, and histiocytes
    - Most commonly in **extremities**
    - Clinical:
      - **<u>PainLESS</u>** mass of several month's duration
      - **Destructive** lesions of **cortical bone**
      - <u>New bone</u>: either tumor or reactive sclerosis
      - X-ray:
        - **Codman's Triangle** (elevated periosteum, *fig*)

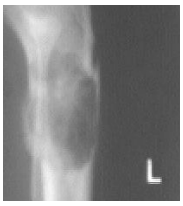




        - Sunburt pattern (radiating spicules)
    - Pathology
        - **Lace-like osteoid deposition** (see *fig*)

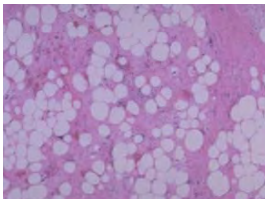


- **<u>Ewing's Sarcoma</u>**
    - **Small** and **round blue cell** tumor (possibly neuroectodermal)
    - Genetics: EWS-FLI1 gene fusion
    - Clinical:
        - **<u>PAINFUL</u>** mass
        - Constitutional symptoms: fever, anemia, leukocytosis
        - X-ray: **Focal** lucency (*whereas osteosarcoma= diffuse*)

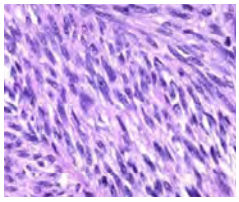


    - Pathology
        - **"Onion skin" periostitis**
- Therapy for PEDIATRIC sarcomas:
    - **CHEMOTHERAPY** ± surgery

- **Adult Sarcomas**
    - **<u>Liposarcoma</u>**
        - Morphologies
            - <u>Pleomorphic</u>: variable tumor morphology
            - <u>Well-differentiated</u>: fat droplets in fibrous bg (see *fig*)
            - <u>Poorly-differentiated</u>: no fat droplets
            - <u>Myxoid</u>: ?



    - **<u>Fibrosarcoma</u>**
        - Compact fascicles of **spindled** cells w/ uniform features (*fig*)



    - **<u>Leiomyosarcoma</u>**
        - **Spindled** cells with directionality
        - Identify with immunohistochemical staining
    - **<u>High-Grade Undifferentiated Pleomorphic Sarcoma</u>**
        - Formerly known as *Malignant Fibrous Histiocytomas* (MFH)
        - Aggressive
        - Undifferentiated, as thus will not express markers of any differentiated mesenchymal tissue
    - Therapy for ADULT sarcomas:
        - **SURGERY** ± chemotherapy
- <u>Genetics of Sarcomas</u>
    - **Simple (Translocation Related)**
        - Characterized by **gene fusion** and/or **chromosomal translocation** that results in formation of *novel protein* that **blocks differentiation**
        - Example: PAX3-FKHR fusion→ Rhabdomyosarcoma
    - **Complex Karyotype**
        - **Most of the adult sarcomas**
        - Characterized by DNA deletions (p53, pRB), amplification of oncogenes (myc), aneuploidy
- Bone Sarcomas
    - Most bone tumors in people >40yo are **metastases** from other organs (lungs, prostate, etc)

    - Primary bone tumors metastasize to non-bone structures—i.e. LUNGS
- Sarcomas of Unknown Origin
    - Ewing's Sarcoma, Clear Cell Sarcoma, Synoval Sarcoma→ unknown lineage or origin

Lecture #17: 11/30- Anticancer Pharmacology I

- Anticancer Drug Production
  - Median time from synthesis to FDA approval is 16.2 yrs for anticancer agents—longer than for other agents (i.e. cardiovascular)
  - Anticancer agents have **lowest success rate** for approval
- **Anticancer Drug Pipeline**
  - **Phase 1**
    - In anticancer trials, Phase I done in cancer patients
    - Determine ***minimum therapeutic dose*** (MTD)
      - Initial: based on one-tenth lethal dose in animal models
      - Stepwise increase in dose until unacceptable SE seen
      - Essential because anticancer agents have **narrow therapeutic window** (i.e. small difference b/w therapeutic dose and toxic dose)
    - Define **pharmacokinetics** (ADME) & **-dynamics** (drug effects)
  - **Phase 2**
    - Single institution
    - Determine efficacy in different tumor types
    - Maximize chance of detecting clinical response
  - **Phase 3**
    - Large (hundreds of patients), multi-institutional
    - Randomized
    - Control group receives SOC + placebo
  - **Phase 4**
    - Post-marketing
    - Adverse events reporting
    - Developments and new indications
  - FDA Endpoints
    - **Time to progression** (TTP): reducing tumor growth
    - **Survival**: mortality benefit
    - **Relief of symptoms**: better quality of life
    - **Delay of event**: prevent complications
- Pharmacokinetics
  - Steady State (SS): rate in (maintenance dose) = rate out (clearance)
  - Volume of distribution (VD): theoretical concept designed to fit time-concentration curves for drug
    - Increased by plasma protein binding (results in seemingly reduced drug efficacy, because higher concentrations are required→toxicity?)
- Pharmacogenetics
  - **Polymorphisms** (SNPs) in **drug-metabolizing enzymes (CYPs)** can lead to individual variability in drug efficacy, toxicity, risk of drug-drug interactions
  - Goal of pharmacogenetics: individualized therapy with genotyping!
- Anticancer Agents
  - Thiopurines

        - **6-Mercaptourine**
            - Indication: for children with ALL
            - MOA: incorporate cytotoxic thioguanines into DNA
            - Metabolized and inactivated by **TMPT** (thiopurine methyltransferase), for which polymorphisms exist
                - Rapid metabolizers: resistant to 6-MP; require higher dose
                - Slow metabolizers: risk for neutropenia; require smaller dose
    - Tamoxifen, Antipresseants, and Anastrazole
        - **Tamoxifen**
            - Indication: ER+ breast cancer
            - MOA: estrogen receptor antagonist
            - **CYP2D6** catalyzes formation of tamoxifen's active metabolite: **endoxifin**
            - Certain CYP2D6 SNPs increase or decrease this activity
            - Antidepressants that act as CYP2D6 inhibitors (ex. paroxetine) prevent formation of endoxifin, and thus inhibit tamoxifen's efficacy
    - Angiogenesis Inhibitors
        - **Bevacizumab**
            - Indication: metastatic breast, colonic, renal cancer
            - MOA: antibody against circulating VEGF, preventing tumor angiogenesis
        - **Sorafenib**
            - Indication: hepatocellular and renal cell carcinoma
            - MOA: inhibits cell surface and intracellular kinases in angiogenic pathway
        - **Sunitinib**
            - Indication: renal cancer or imatinib-resistant GIST
            - MOA: inhibits multiple RTKs, including those in angiogenic pathway
    - Chemotherapy
        - **Anthracyclines: Doxorubicin**
            - MOA: intercalates DNA
            - SE: cardiotoxicity
        - **Taxanes: Paclitaxel, Docetaxel**
            - MOA: bind tubulin and stabilize polymerized microtubules, preventing cell division
            - SE: hypersensitivity, myelosuppression (cytopenias)
        - **Platinum Coordination Complexes: Cisplatin, Oxaliplatin, Carboplatin**
            - MOA: cross-link DNA
            - SE: nephrotoxicity (ATN), ototoxicity
        - **Vinca Alkaloids: Vincristine**

        - MOA: binds tubulin and blocks polymerization of microtubules
        - SE: myelosuppression, neurotoxicity
    - **<u>Alkylating Agents</u>: Cyclophosphamide**
        - MOA: mustard-type DNA alkylating agent which results in cytotoxicity via DNA cross-linking
        - SE: pulmonary fibrosis, cardiotoxicity, amenorrhea
    - **<u>Antibiotics</u>**: **Bleomycin**
        - MOA: induced formation of free radicals→ DNA breaks
        - SE: <u>pulmonary fibrosis</u>

Lecture #18: 11/30- Anticancer Pharmacology II

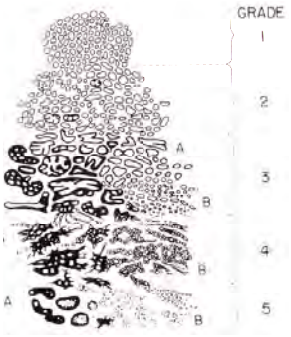
- About **one-third** of cancer patients are **cured** with local treatments such as **surgery** and/or **radiation** therapy
- **Chemotherapy:**
    - Only 10% of all cancer patients are cured by chemotherapy
    - Roles
        - **Adjuvant**: after surgical resection to prevent relapse
        - **Neoadjuvant**: before surgery, or for non-operable tumors
        - **Combined:** treatment for locally advanced dz; chemo + RT
        - **Primary Chemotherapy**: treatment of metastatic disease
- Cancer Kill Kinetics
    - Skipper & Schabel Model
        - Single cancer cell can give rise to sufficient progeny to kill host
        - Anticancer agents kill cancer via first order kinetics (fixed %)
        - Host immune mechanisms play negligible role in cancer kill
    - Gompertzian Model
        - Tumor ***<u>growth fraction</u>*** (fraction of cells actively progressing through cell cycle) peaks when it is 37% of maximal size (when chemotherapy works best) and then decreases exponentially
        - **Chemotherapy kills cells that are actively growing** (earlier)
        - Thus, more advanced cancers are less responsive to chemo
- Combination Chemotherapy
    - Use of multiple chemotherapy agents
    - Required for cancer cure
    - Best to use drugs that have different MOAs
    - Prevents or slows down development of drug resistance
- Limitations of Cancer Chemotherapy
    - Difficult to asses presence of *minimal residual disease* post treatment
    - Associated with dose-dependent toxicity; narrow therapeutic window
    - Low specificity of effect (i.e. tumor vs. normal tissue)
    - Cycling vs. non-cycling cancer cells
    - Development of resistance
- Targets
    - **Epidermal Growth Factor Receptor (EFGR)**

        - Inhibition of EGFR in EGFR+ tumors inhibits growth and progression
        - Monoclonal antibody: block ligand binding
            - **Cetuximab**: anti-EGGFR antibody for metastatic colorectal cancer
        - Tyrosine kinase inhibitors: block RTK activity
    - **HER-2 Receptor (HER2R)**
        - Monoclonal antibody
            - **Trastuzumab**: anti-HER2/neu antibody for HER2+ metastatic breast cancer

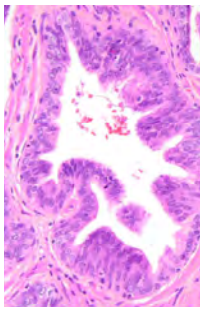
Lecture #19: 12/1- Prostate Cancer (TBL)

- Development of Prostate Cancer
    - Normal prostate epithelium→ prostatic intraepithelial neoplasia→ localized prostate cancer→ metastatic prostate cancer→ castration resistant cancer
- Epidemiology
    - Age: prostate cancer is a disease of **aging** (~70s)
    - Race**: African Americans** have higher incidence and mortality
    - Diet: **Western** (vs. Asian) diets associated with higher incidence
    - Overall, PCa incidence is increasing and mortality if falling
    - **Early stage** PCa is **detectable** with DRE and PSA, and is **curable**
- Screening
    - Digital rectal exam (DRE) is extremely valuable test for finding PCa
    - Studies indicate that there is a **mortality benefit** from **PCa screening** (vs. non-screened controls) **13 years after** initiation of screening (*controversial*)
    - **Prostate Specific Antigen (PSA)**
        - PSA is a serine protease that is **secreted by prostatic epithelial cells** and is **normally found in blood**
        - Normal level varies with individuals, but usually **<4 ng/mL**
        - In PCa, proliferation of prostatic epithelial cells results in increased level of serum PSA
        - However, its use as a screening test is **CONTROVERSIAL** because it is not cancer-specific; elevations in PSA can occur in response to age, prostatic massage, BPH, prostatitis, instrumentation, etc.
        - Thus, better to look at **PSA refinements**:
            - Free/Total PSA:
                - PSA produced by PCa tends to be **protein-bound**, so lower ratio points to malignancy
            - PSA Velocity:
                - Analysis of the rate of increase in PSA level over time; rapid increase indicates malignancy
- Pathology

- PCa is an **adenocarcinoma** that arises in **peripheral zone** of prostate epithelium (this region is palpable on DRE)
- **High-Grade Prostatic Intraepithelial Neoplasia (HGPIN)**
  - Precursor lesion
  - Multilayered epithelial cells (see *fig*)
  - Basal cell layer present
- Prostatic Carcinoma
  - Crystalloid secretions in glands (well diff.)
  - Prominent nucleoli
  - Loss of glandular architecture with pallisading cells (poor diff.)
- Metastasis
  - Lymphatogenous: obturator→para-aortic nodes
  - Hematogenous: **<u>bones</u>** (spine, femur, pelvis, ribs)
- **Gleason Grading for Prostatic Carcinoma**
  - <u>Grades</u>: 1 (well-differentiated)→ 5 (poorly differentiated)
  - <u>Gleason Score</u>: summation of 2 Gleason grades, based on 2 most common tumor patterns in patient's specific biopsy
    - Differentiation based on score:
      - 5-6 (moderate), 7 (moderate-poor), 8-10 (poor)
  - *It is not until high Gleason scores that men begin to die of their prostate cancer rather than of unrelated causes*



- Treatment
  - Localized Disease
    - **<u>Radical Retropubic Prostatectomy (RRP)</u>**
      - SE: injury to **neurovascular bundle**: can result in impotence/erectile dysfunction (79.6%)
      - Careful urethral re-anastomosis: urinary continence
      - Results in high *progression-free survival*, **but overall survival benefit not proven**
      - **After prostatectomy, PSA should be <0.01 ng/mL**
        - 2 successive increases → **recurrence**!
    - **<u>Watchful Waiting (WW)</u>**
      - Indicated in **older men** who are asymptomatic, as survival is equal to those who receive prostatectomy
    - **<u>Radiation Therapy (RT)</u>**
      - Can use *intensely modulated radiation therapy* (IMRT) to specifically target areas of more cancer and minimize side effects of RT
      - SE: *radiation proctitis* (19%), impotence (62%)
    - **<u>Brachytherapy</u>**
      - Implantation of "**radiation seeds**" into prostate cancer
      - No evidence says it's better than external beam radiation
  - Locally Advanced Disease
    - **<u>Androgen Deprivation Therapy</u>**



- Prostate epithelial cells are dependent on androgens for survival; if deprive of androgens, cells stop growing
- 1) **Orchiectomy**
  - Removal of the testicles (main androgen source)
- 2) **GnRH Agonist** (Leuprolide)
  - +GnRH→+LH→+androgens→ *less* androgens over time via **negative feedback** on LH
- 3*) Finasteride (FOR BPH & balding, not PCa)*
  - *Blocks conversion of testosterone to dihydrotestosterone*
  - *SE: +**hair growth** in male-pattern balding*

▪ **<u>Combined: Radiation + GnRH Agonist</u>**
- RT + Goserelin: large survival benefit seen

o Metastatic Disease
▪ Common site of metastasis
- **BONE**→ manifests as pain, particularly spine

▪ *Must consider morbidity of androgen ablation*
- *Osteoporosis, fatigue, diabetes, cardiovascular risk*

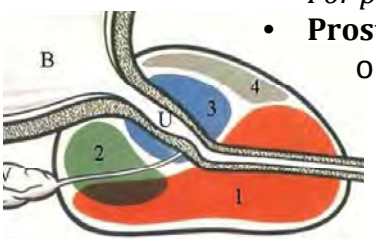
▪ **<u>Early Androgen Ablation + Surgery</u>**
- Increased survival with total androgen ablation, including **adrenal androgens,** in *<u>poorly differentiated cancers</u>*
- In metastatic disease, androgen ablation therapy should not be d/c, even after progress on GnRH agonist

o Castration Resistant/Androgen-Independent Disease
▪ Cancer develops androgen receptor mutations which amplify it's activity or which increase sensitivity to cancer's own androgen production
▪ **Second-line hormonal therapy**
- Ketaconazole, steroids

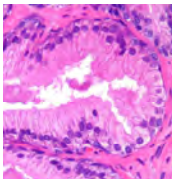
▪ **<u>Chemotherapy (in hormone-refractory cases)</u>**
- **<u>Docetaxel</u>**
  - Only chemotherapy agent that has proven successful in treating prostate cancer
  - Stabilized microtubules so that cell division does not take place

Lecture #20: 12/1- Prostate and Testicular Pathology

- *For prostate cancer, refer to Lecture #19*
- **Prostate**

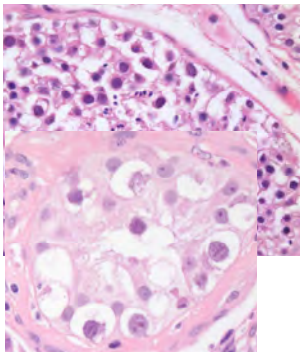


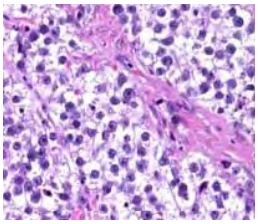
o Anatomy
▪ Zones (refer to *fig*)
- 1) **Peripheral** (Prostate Cancer)
- 2) Central
- 3) **Transitional** (Benign Prostatic Hyperplasia, BPH)
- 4) Anterior Fibromuscular

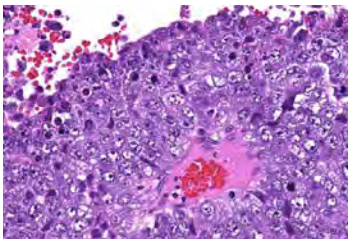


- Histology
  - Lobular configuration of **ducts** and **glands** (with apical-basal organization)
  - **Growth** of prostate cells is **androgen-dependent**
- Pathology
  - **Benign Prostatic Hyperplasia**
    - Very common (70% men >60 years)
    - Hyperplasia of stroma/glands in the **transitional zone** resulting in **formation of large nodules** in the **periurethral region**
    - Nodules can cause partial or complete **urethral obstruction**
    - Clinical:
      - **Urinary retention**, **hesitancy**, **frequency**; nocturia; dysuria; urinary tract infections
    - Therapy
      - **Alpha Blockers**: decrease prostate smooth muscle tone via alpha 1 adrenergic blockade
      - **5-Alpha-Reductase Inhibitors (Finasteride)**: inhibit conversion of testosterone into dihydrotestosterone→ androgen
      - **Transurethral Resection of Prostate** (TURP)

- **Testicles**



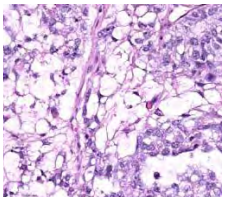
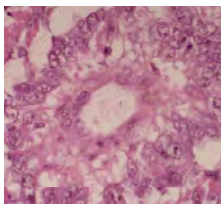
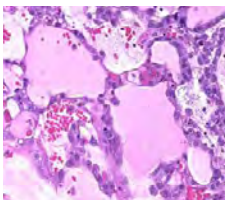




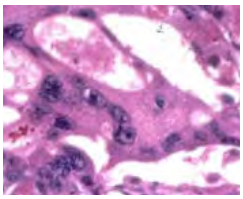
  - Normal histology: see *fig*
  - Malignancy can arise in testes (below) or paratestes (not included)
  - **Testicular Tumors**
    - Germ Cell (95%)
      - *Risk factors: environmental, **cryptorchidism**, familial*
      - **Seminomas**
        - 50% of germ cell tumors
        - Peak incidence: 30s
        - Precursor Lesion: **intratubular germ cell neoplasia** (ITGCN)
          - Occurs *in utero*; dormant until puberty
          - Atypical germ cells with large nuclei, clear cytoplasm, prominent nucleoli (fried-egg)
        - Microscopically:
          - Sheets of cells divided by **fibrous septae** into poorly demarcated lobules
      - **Non-Seminomas**
        - **Embryonal Carcinoma**
          - Peak Incidence: 20-30 y
          - **Aggressive** tumors with **hemorrhage** and **necrosis**
          - Microscopically:
            - Alveolar, tubular, or papillary

- Cells pleomorphic, highly atypical
    - Mitotic figures

- **Yolk Sac Tumor**

  - Most common in infants and <3 yo's
  - Serum marker: **alpha fetoprotein** (AFP)
  - Microscopically: (see *fig*)
    - **Lace-like (reticular) network**
    - **Schiller Duval bodies**
    - **Hyaline-like** globules (AFP, A1AT)

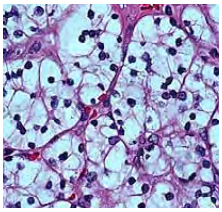


- **Choriocarcinoma**
  - Serum marker: **HCG**
  - Hemorrhage, necrosis
  - Microscopically:
    - **Syncytiotrophoblasts**→ HCG
      - Large cell w/ ++ nuclei (*fig)*
- **Teratoma**
  - Complex tumors with differentiated cells exhibiting components of normal tissues
  - Mature form: resembles adult tissue
    - In prepubertal males→ benign
  - Immature form: resembles fetal tissue

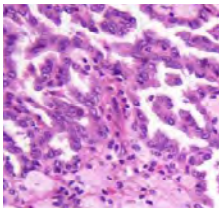
- Spread:
  - Lymphatic→ para-aortic nodes
    - Seminomas
  - Hematogenous→ lungs
    - Non-Seminomas

Lecture #21: 12/2- Renal and Bladder Pathology

- **Renal Neoplasms**
  - **Renal Cell Carcinoma**
    - Adenocarcinoma of the kidney
    - Epidemiology
      - M > F
      - Risk factors: smoking, obesity (women), HTN, CRF
      - Most are sporadic
    - Clinical Symptoms
      - **Costovertebral pain**, hematuria, fever, malaise, weight loss
    - Classifications

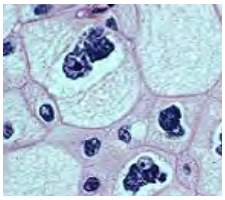


      - **Clear Cell Carcinoma**
        - Most common (70-80%)
        - Origin: proximal tubule
        - Solitary
        - Solid nests of clear cells; well-differentiated



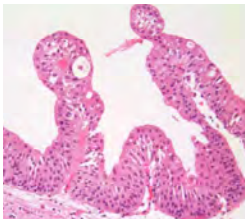
      - **Papillary Carcinoma**
        - 2nd most common (10-15%)

        - Origin: distal convoluted tubule
        - Multifocal, bilateral
        - Trisomy 7, 16, 17
    - **<u>Chromophobe Renal Carcinoma</u>**



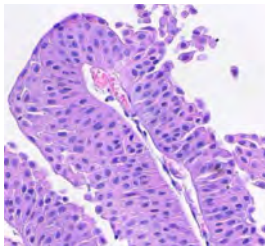
        - 5% of RCC
        - Origin: intercalated cells of collecting duct
        - Hypoploidy
        - "Raisinoid" nuclei
        - Excellent prognosis
    - **<u>Collecting Duct Carcinoma</u>**
        - 1% of RCC
        - Origin: collecting duct cells in medulla
        - **Associated with sickle cell disease/trait**
        - Poor prognosis (most aggressive RCC)
- **<u>Urothelial Carcinoma</u>**
    - Arises from **transitional epithelium**
    - Clinical Symptoms
        - **Obstruction→hydronephrosis,** hematuria, flank pain
    - "Field Effect": can be <u>multifocal</u> (bladder, kidney, both kidneys)
- Wilms Tumor
    - Refer to *pediatric oncology* lecture

- **Bladder Neoplasms**
    - **<u>Urothelial Carcinoma (95%)</u>**
        - Transitional Epithelium
            - Umbrella cell layer (apical), intermediate layer, basal cell layer
        - Epidemiology
            - M:F = 3:1
            - Age range: 50-80 yo
            - Risk factors: **smoking**, exposure to arylamines, schistosoma haematobium, radiation (prostate ca)
        - Genetics
            - Deletion of tumor suppressor genes on **9p and 9q→** superficial tumor→ additional **p53** mutation→ invasive
            - *Vice versa*
        - Clinical Symptoms
            - Bladder tumors produce **painless hematuria**
        - <u>Precursor Lesions (in situ)</u>

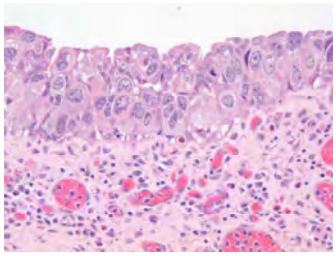


            - (1) **Papillary Neoplasm (most common)**
                - <u>Papilloma</u> (see *fig*)
                    - Younger pts
                    - Single and small
                    - Papillae with **normal urothelium**
                    - Rarely recur
                - <u>Inverted Papilloma</u>

        - Similar to papilloma but **urothelium grows endophytically** (towards)
    - <u>Papillary Urothelial Neoplasm of Low Malignant Potential (see *fig*)</u>
        - Like papilloma, except urothelium is thicker (10-15 layers)
        - Can recur
        - Rarely progress to carcinoma
    - <u>Papillary Urothelial Carcinoma, Low Grade</u>
        - Disorganized urothelium with variable mitotic activity
        - Recur and progress (to high grade)
        - Rarely invade
    - <u>Papillary Urothelian Carcinoma, High Grade</u>
        - Disorganized urothelium, diffuse mitotic activity, hyperchromasia, **nercrosis**
        - Recur and progress
        - **Invade**



- (2) **Flat Urothelial Carcinoma In Situ** (see *fig*)
    - Unlike exophytic growth with papillomas, FUCIS is flat and difficult to see on cystoscope
    - Disorganized urothelium, diffuse mitotic activity, hyperchromasia, **nercrosis; granularity**
    - **Invade** (50-75%)

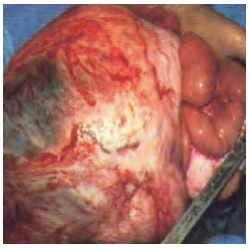


- Invasive Urothelial Carcinoma
    - 50% of patients present with invasive disease
    - **Muscularis propia** invasion = poor prognosis
- Treatment
    - Transurethral Resection
        - Papillary urothelial carcinoma, low grade
    - Intravesicular chemotherapy
        - In Situ Carcinoma and PUC, high grade
    - Radical Cystectomy
        - Muscular propia invasion

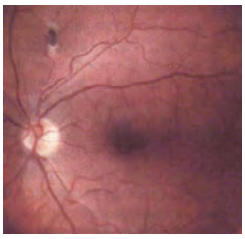
Lecture #22: 12/2- Colon Cancer

- Epidemiology
    - 3rd most common cancer in U.S.
    - Incidence rates high in western countries, increasing in Japan
        - Suggests environmental factors: DIET!
- Risk Factors
    - Protective
        - Exercise
        - **NSAIDs**
            - <u>Non-selective</u> COX inhibitors→ **bleeding** risk!
            - <u>Selective COX-2</u> inhibitors→ **thrombosis** risk!

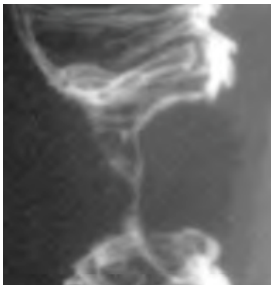
        - *As such, NSAIDs **NOT** recommended for CRC prevention*
    - Increased Risk
        - **Advanced age**, IBD, high-fat diet, personal/family history
- Pathogenesis
    - "Multi-hit hypothesis" says multiple genetic changes are necessary for cancer development; demonstrated by APC and HNPCC syndromes
    - Genetic Model of Cancer Progression
        - Normal epithelium→(1st hit: *APC mutation*)→ adenoma→ (2nd hit: *p53/PTEN mutation*)→ carcinoma→ metastasis
    - **Hereditary Non-Polyposis Colon Cancer** (HNPCC, *Lynch Syndrome*)
        - Autosomal dominant
        - 2-5% of colon cancers
        - Mutation in mismatch repair gene
        - Presentation: **right colon**; associated with <u>endometrial cancer</u>
        - Screening: **start in early 20s**



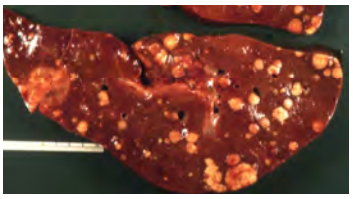
    - **Adenomatous Polyposis Coli (APC)**
        - Autosomal dominant
        - <1% of colon cancers
        - Mutation of APC gene (beta-catenin disinhibited)
        - Associated with duodenal/ampullary cancers, **desmoid tumors** (*fig*), ***congenital hypertrophy of the retinal pigment epithelium***, CHRPE (*fig*)
        - Screening: **start at puberty**



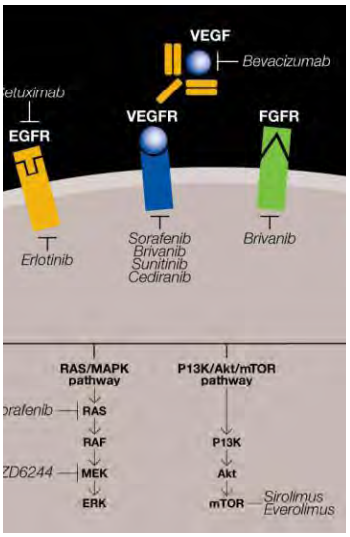
- Screening
    - Detection via screening:
        - 1) Fecal occult blood
        - 2) Sigmoidoscopy
        - 3) **Colonoscopy**→ <u>GOLD STANDARD</u>
        - 4) Virtual colonoscopy
    - Screening Protocols
        - <u>Average risk</u>: colonoscopy every 10 yrs after 50 yo
        - <u>Family history</u>: colonoscopy 10 yrs before index case
        - <u>APC</u>: sigmoidoscopy at 11 yrs, polyps→ colectomy
        - <u>HNPCC</u>: colonoscopy at 21 yo, then every 1-2 yrs
        - <u>IBD</u>: 8 yrs post pancolitis, 12 yrs post distal disease
- Manifestations
    - Local Disease
        - Asymptomatic
            - Detected with screening
        - **<u>Right-Sided Syndrome</u>**
            - Ascending colon→ thin, distensible wall; wide
            - Liquid fecal stream
            - Chronic, <u>occult</u> blood loss results in **iron deficiency anemia**
                - Blood rarely noticed in stool
            - Obstruction unlikely



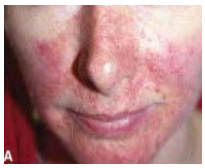
        - **<u>Left-Sided Syndrome</u>**
            - Descending colon→ thick, less distensible; narrower
            - Solid fecal stream
            - **Bright red blood per rectum** more common
            - **Obstruction** more common
        - *"Apple Core" Lesion*
            - Annular constricting lesion of the colon leading to luminal narrowing (see *fig*)

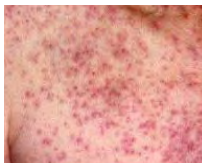


    - Metastatic Spread
        - Lymphatogenous:
            - Mesenteric nodes, **Virchow's node** (L supraclavicular)
        - Hematogenous
            - **LIVER** mets (pain, hepatomegaly, +LFTs)
- Staging
    - Stage I
        - Cancer grown through mucosa and **reached muscularis**
        - Rx: surgical resection (including lymph nodes)
        - Survival: 90%
    - Stage II
        - Cancer grows **beyond muscularis** but not to lymph nodes
        - Rx: Colon: surgical resection (± adjuvant chemo)
        - Rx: Rectum: surgery, radiation and chemo
        - Survival: 70-85%
    - Stage III
        - Cancer spread to regional lymph nodes
        - Rx: Colon: surgery **+ adjuvant chemotherapy**
        - Rx: Rectal: surgery, radiation, and chemo
        - Survival: 40-80%
    - Stage IV
        - Cancer spread to other organs (esp. LIVER)
        - Rx: chemotherapy; surgery to remove mets in selected patients
        - Survival: evolving
- Treatment
    - Surgery
        - Localized disease (stage 1, 2, 3)
    - Radiation
        - Rectal cancer (to prevent local recurrence)
    - Pharmaceuticals
        - Stage III (node +) and IV
        - **5-Fluorouracil**
            - MOA: pyrimidine antimetabolite (→dec. dTMP)
            - SE: **cerebellar toxicity**, N/V, diarrhea, mucositis
        - **Irinotecan**
            - MOA: topoisomerase inhibitor (prevents DNA religation during synthesis)
            - SE: **alopecia**, anemia, weight loss



- **Oxaliplatin**
  - MOA: alkylating agent
  - SE: cold intolerance, paresthesias
- **Bevacizumab**
  - MOA: antibody against VEGF: blocks angiogenesis
  - SE: bleeding, thrombosis, HTN, asthenia, proteinuria
- **Cetuximab, Panitumomab**
  - MOA: antibody against EGFR: prevents dimerization
  - **Efficacy blocked by downstream mutations in <u>K-RAS</u>**
  - SE: **dermatologic eruption** (rash)→ indicates drug is working; correlated with efficacy (see *fig*)





- Metastatic Disease
  - Systemic chemotherapy→ improved survival to 2 years
  - Resectability of liver metastases determined by:
    - 1) Can <u>all</u> mets be resected?
    - 2) Sufficient hepatic remnant (i.e. at least 20%)
  - **<u>OCCASIONAL CURES POSSIBLE</u>**