Notes from DNA Methylation lecture (from 2012 lecture)

DNA methylation plays a role in differentiation, mutageneisis, X inactivation, development of some cancers, and other epigenetic changes in response to the environment

Epigenesis: modifications to genes other than changes in the DNA sequence; usually due to methylation. These modifications to the DNA can be preserved and passed down during cell division to daughter cells.

Epigenetics: inheritance from cell to cell or organism to organism by changes in chromatin &/or DNA but not in the DNA sequence (from Dr. Ehrlich's slide)

- extensive cell-type specificity, e.g., skeletal muscle & brain
    - changes with normal cell physiology : e.g., brain function ; skeletal muscle function
- changes with aging
- changes with environment
- inter-individual differences
- changes involved in disease, e.g., cancer, autoimmune diseases, & many others

There are 2 types of epigenetics:

1. **DNA methylation**. most commonly involves the attachment of a molecule called a "methyl group" to cytosine. When methyl groups attached to the DNA, these genes are usually turned off. These marks are usually passed to daughter cells. (Most of the lecture was on this type of epigenetics)
2. **Chromatin Epigenetics** - Mostly histone modifications or histone content. Affects the packaging of chromatin. Cell-type specific modification. Not sure if it gets passed to progenitor cells. Wide variety of modifications.

How does DNA become methylated? - after DNA synthesis

- Predominate site is CpG site (she talk about direction, but you don't need to know)
- The symmetrical pattern of the nucleotides is why the methylation can be maintained and "passed down" methylation pattern ("Maintenance methylation")
- Establishment of most methylation patterns occurs during embrogenesis
- There is a difference between Maintenance methylation and De novo Methylation: Maintenance methylation is related to keeping genes turned off and differentiation
- 3 types of enzymes involved:
    DNMT1 (main enzyme for maintaining methylation)
    DNMT3A (especially important for *de novo* methylation)
    DNMT3B (especially important for *de novo* methylation)

Facts about DNA methylation: (and its role in embreogenesis, differentiation and mutagenesis)

- Increased methylation of promoter regions are associated in general with decreased expression of the gene
- So this down-regulation of genes is probably responsible for the role of methylation in establishing some types of differentiation
- From knock-out mice, seems essential for life (loss of methylation is lethal in mice)
- Changes with aging
- About a third of human disease is estimated to be related to mutations in CpG because 5mCpG's seem to be Hot spots for spontaneous mutaenesis – esp. in P53 genes related to cancer

Patterns of DNA methylation:

- Vertebrate genomes are depleted in CpG
- CpG islands often at promoters – small patches in genome, when most of genome is lacking
- Helps identify promoters (60% have CpG islands)
- Most promoters are unmethylated
- If there are CpG islands overlapping promoters – they are often housekeeping genes (need to be on most of the time),

Different Roles of methylation:

1) **Differentiation** – tissue specific methylation
    - Important in spermatogenesis and oogenesis
    - Essential for mammalian development
    - At implantation surge in de novo methylation

2) **X- inactivation**: Inactivation of second X chromosome in females is related to changes in DNA methylation – the ACTIVE X has a gene (XIST) that is methylated and therefore turned off that protects the rest of that X from getting inactivated (Ironically, you inactivate a gene to prevent inactivation of the chromosome – at least that is how understand it

3) **Imprinting and Disease:**
    - **Fragile X** –increased methylation of a CpG island accompanies the increase in the number of copies of the triple repeat in Fragile X
    - Methylation is often related to imprinting
    - Maternally expressed genes (only mom's genes expressed) – usually suppress growth. Paternally expressed genes usually enhance growth (don't need to know, just an interesting fact) An example of this is Beckwith-Wiedermann syndrome in which epigenetics lead to only paternal genes working and abnormal overgrowth
    - **Prader-Willi** – If you recall, PW is caused by either a deletion of the paternal gene, uniparental disomy of the maternal gene OR a mutation in the methylation gene region such that the paternal gene is not expressed. In all cases, Dad's gene is not being expressed

4) **Cancer and Tumor development:**
    - Major source that promote cancer formation and tumor progression is the abnormal de novo methylation of promoter regions of genes with an anti-tumor function (tumor suppressor genes). So the genes that are suppose to suppress tissue growth are turned off, so tumors develop. This is a major contributor to tumor progression
    - However, in tumor formation both genomic **hyper** and **hypo**-methylation is observed
    - Hypermethylation has been correlated with cigarette smoking (an example of environmental factors affecting the genome)
    - Example; BRAC1 and BRAC2 – inherited 1 copy of predisposing mutation, the second copy is often deactivated by hypermethylation (sourse of the "second hit" for tumor growth)
    - So – looking at the epigenome is important in prognosis in many cancers
    - Some cancer chemotherapy target epigenetic changes in cancer, although these currently are not successful for solid tumors and most types of leukemia

- Epigenetic therapy to decrease methylation wholesale does not work because hypomethylation may cause its own problems – need patient-specific/ tumor specific- treatment

5) **Negatively regulating (Turning off) expression of foreign DNA:** (this characteristic is relevant in affecting gene therapy – making it more difficult)
   - Foreign DNA
   - Transposable elements
   - Some viral DNA
   - Proviruses

6) **Other Epigenetic effects:**
   - In vitro fertilization results in higher rate of imprinting abnormalities – probably related to abnormal methylation during embryogenesis
   - Cloning animals – problem in cloning is birth defect related to DNA methylation re-programming is disrupted in this process
   - Some epigenetic changes are not just on/off switched but are modulators of efficiency of keeping genes turned off.

7) **Autosomal Recessive Disease related to Methylation**: Human disease known to involve Mendelian inheritance of abnormal DNA methylation: ICF syndrome. Caused by mutation in one of the DNA methyltransferase genes

8) **Other notes:**
   - Epigenetic mistakes more frequent than inherited mutation
   - Therapy: some work to try to decrease methylation levels.
   - Epigenetic changes have a lot to do with turning genes on and off in a way that can be preserved and even 'passed down"

Dr. Ehrlich's last slide - summary (easier to read form)

- Epigenetics: changes in enzymatically produced DNA methylation, hydroxymethylation, or inherited changes in chromatin structure, especially, histone modification
- Most human promoters have CpG-rich, unmethylated DNA
- DNA methylation (5mC) at promoters or enhancers usually downregulates transcription
- 5-hydroxymethylcytosine (5hmC) is a newly discovered base in DNA that can help control transcription
- 5mC and 5hmC DNA residues are essential for normal function in mammals
- Epigenetic changes are key to normal development in mammals
- Cancers usually have hypermethylation (increased 5mC) at some tumor suppressor genes in the promoter regions and much hypomethylation (decreased 5mC) in other parts of the genome
- Hypomethylation and hypermethylation of DNA in cancers are important for their formation and tumor progression and can be diagnostic and prognostic tools.
- Some cancer chemotherapy target epigenetic changes in cancer, although these currently are useful only for a small subset of neoplasms (e.g., DNA hypomethylating agents in myelodysplastic syndrome & secondary acute myeloid leukemia).