Module 1: Lecture One

- What is science
  - Science is constantly changing and evolving whether for better or for worse
- Characteristics of science
  - Main goal of science is to come up with a general principle that is universally applicable
- 1. Empirical knowledge
  - Initial observation is usually the first step if the scientific method
- 2. Rational/Natural explanations for phenomena
  - Some kind of explanation behind the phenomenon
  - Science cannot explain supernatural events
- 3. Repeatable and Reproducible
  - Science is reliable when the experiment can be repeated with the same results
  - Science does have restraints that may not allow the experiment to be run again
    - Ie. If the experiment is costly, requiring a lot of ppl,
  - There are limits to the conclusions we make and usually they are technical restraints
- 4. Testability
  - For the hypothesis to be called as such it MUST be testable
- 5. Experimentation
  - Applies some kind of manipulation of a system
- 6. Generality of Principles
  - Ie. If the experiment is done on males then the principle can only be generalized to males and not the whole human population

Module 1: Lecture Two

- Case study: human Chromosome Count
  - Science is not perfect bc it is done by scientists who are also prone to human error and subjectivity
- Case Study: Human chromosome count
  - How we discovered that we were diploid organisms with 23 pairs of chromosomes (46 chromosomes)
  - There were technical limitations to this type of study
  - When you take a chunk of a cell you can't control the orientation of the chromosomes so you might end of cutting it in half and counting it twice in two different sections
- Repeatability/ Reproducibility
  - Testicular tissue wasn't the best tissue to work with bc it had both diploid cells as well as sperm cells which are haploid
  - For many years we thought we had 48 chromosomes, but this is wrong
- 1950's - Improvement of Techniques
  - Main differences this time
    - Flattening the cell so that you wouldn't have to serial section
    - Using different types of solutions that prevented fragmentation of chromosomes
    - Colchicine was used

        - This inhibits spindle formation which would allow the chromatids to stay together rather than be pulled apart by the spindles
    - Using the tissue right away
        - If you let the cell sit and chill the DNA would eventually degrade or chromosomes would fragment
    - More use of ideograms
- 46: The truth revealed
    - We actually have 46 chromosomes which was discovered years after the statement that we had 48 chromosomes
    - All the improvements made on the technique allowed them to refine their discovery and find the truth
- So what (if anything) went wrong in Painter's approach?
    - Sample source: Painter was working with SPERMATOGONIAL samples not sperm samples
    - Painter did his best with what he had
    - Taking samples from different places will strengthen your results

Module 2: Lecture One

- Characteristics of SCs
    - Because they are unspecialized it is usually spherical as it has no specialized regions
- Classification of SCs based on Potency
    - How many times a stem cell can renew depends on the type of stem cell
- Which cell has the most potential?
    - After a few cell divisions of a zygote it forms the morula
        - The morula also has totipotent stem cells
- Sources of ESCs
    - Take a somatic cell that is diploid
    - Isolate the nucleus
    - Take an egg and remove the nucleus
    - Then inject the diploid nucleus into the egg

Module 2: Lecture 3

- Identifying the yamanaka Factors
    - They used genetically modified fiberglass with this experiment
    - They affected cells with viruses and cultivated them
    - Identified four crucial factors that allows a pluripotent cell to be a pluripotent cell
        - The cells were able to colonize with all four factors
        - It was resistant to antibiotics
            - This shows that the cells became pluripotent
    - The moment one of the factors was taken away it doesn't colonize as well
        - Less than half
    - Having just 2 factors is even worse
    - Adding the 10 factors is a positive control
    - The mock was used because there was no factors in it and this was a negative control

    - 10 compared to 4 factors shows that having just the 4 factors is sufficient to have good colony growth
    - This experiment is not telling them us about differentiation potential only about self renewal or proliferation potential
- Are iPSCs the same as ESCs
    - The function of telomerase is to prevent the telomeres from shortening by replicating them
    - Markers are cell surface proteins, cytoplasmic proteins, etc
        - Things that are particular to a specific cell type
    - This paper was a breakthrough bc they were able to produce pluripotent stem cells that weren't embryonic stem cells
        - ESCs have ethical issues but the iPSCs come from fibroblasts so it was fine and easy to find
- Generation of iPSCs - Summary
    - The good thing about iPSC is that we're not using ESC
        - Again the ethical issues
    - They are a source for any kind of differentiated cell
- iPSCs as in vitro model of human disease
    - A lot of research went to trying to find out the causation of microcephaly , if it was due to ZIKV virus or not
    - Some studies suggested that these cells were the targets of ZIKV virus
    - They made iPSC to become human neural progenitor cells and then performed experiments on that
    - They wanted to see if ZIK would attack the hNPC or the neurons which is in the final differentiated cell
    - The experiment showed a decrease in neurons which supported the hypothesis
    - They fail to reject the research hypothesis
- iPSCs-induced organoids as in vitro model to study organogenesis and tissue morphogenesis
    - The different colors represent different cells
    - They are used not to understand the function of an organ but used to understand the organization of the different types of cells or why they're there and how they form
- iPSCs and Cell Therapy
    - Cell replacement
        - Can be used to replace non-functional cells
    - They are grafted to replace the non-functional cell
    - They can potentially come from the same patient which significantly reduces the risk of rejection
        - Taking fibroblasts from the patient
    - Cell therapy and cell replacement protocol has a rule of only grafting partially-differentiated pluripotent SC as to prevent the formation of teratomas
- iPSCs and Cell therapy - Genomic Aberrations
    - CNVs may cause detrimental effects on gene expression

- iPSCs as in vitro model for drug testing
    - Able to use actual human cells for drug testing through iPSCs

Module 4: Lecture 2

- Examples of vertebrate adult SC niches
    - We will be focusing on the intestinal crypt
    - There is research going on about how SC niches behave normally and then how they can be manipulated to be used as a therapeutic thing for chronic diseases such as cancer
- The intestinal Epithelium- Overview
    - 2 main regions (functionally and structurally different)
        - Villi
        - Crypt
    - The crypt is very much in the connective tissue
    - the villi stick out and are mainly made of absorptive tissue and made of enterocytes
        - There are no progenitor or SC
        - Mainly j differentiated cells
    - Whereas the crypt is mainly undifferentiated cells
        - Made out of mainly TA cells (progenitor cells)
            - There is a second type of progenitor cells called secretory cells which give rise to the secretory cells
        - And Stem cells
- Intestinal Stem Cells
    - Intestinal cells have two different types of SC
    - Lgr5+
        - Radiation sensitive
        - Mitotically active
        - It is a genetic marker
    - +4 cells
        - Radiation resistant
        - Not mitotically active
        - Not a marker
        - Positioned a little higher up
- The intestinal Epithelial SC niche - main components
    - The secreted factors are important
- Extrinsic factors influencing IESC activity
    - Wnt and BMP are secreted factors
    - High concentrations of Wnt at the base and low at the top
    - Low concentration of BMP at the base and high at the top
    - This maintains a balance which is necessary for proliferation and differentiation
        - High BMP-Low Wnt
            - Signal to differentiate
        - Low BMP-High Wnt
            - Signal to proliferate

    - If you increase the concentration of Wnt higher up in the crypt then you get more proliferating cells higher up in the crypt
- Role of Delta-Notch Signalling in controlling Intestinal Cell Fate
    - Delta is a lignin for notch
        - Delta is expressed on the surface of a Paneth cell
        - Notch is expressed on the surface of SC
        - It is not secretory factor
        - It has an extracellular domain that interacts with the notch receptor and activates it

- Delta - Notch signalling
    - Activation of the Delta-Notch pathway ends up activating genes such as Hes1
- Inducible Gene expression systems
    - One thing they used was an inducible gene expression system
        - Mice have a P450 1A-Cre which is a promoter and coding region for cre expression
            - The activity of this promoter is stimulated when there are high levels of certain drugs/substances
                - Ie, beta-naphthoflavone
            - The activated complex activates the transcription of the Cre gene
                - Allows for high cre recombinase
        - This whole thing allows for temporal control of gene expression
            - You decide when you want this gene to be expressed bc you have to give the b-naphthoflavone drug
- Gene knockout using cre-loxp recombination system
    - Cre is a recombinase that carries out DNA recombination
    - The cre enzyme deletes whatever's between the loxP sites
    - This gene is then deleted from the genome
    - The mouse is normal until you inject it, then they become mutated due to the activation of the Cre gene which goes about deleting a specific gene
- Disruption of Notch signalling pathway induces goblet cell conversion of crypt proliferative cells
    - They looked at the mice 5 days after injection
    - B and H rep the experimental group
    - A and G are the control groups
    - Decrease in the number of cells that are proliferating
        - Bc they have differentiated into goblet cells already
- Notch and Wnt signal activity in the niche
    - There's still research going on about the different signals
    - Working Ho: a cell that is undifferentiated and is able to proliferate, the wnt pathway and notch control the activity

Module 4: Lecture 3

- Factors affecting the composition of gut microbiota
    - Malnutrition
        - What we eat and what we don't eat
    - Geographic location
        - Westernization of gut microbiota
            - Research about ppl who come from the west to the east
- The gut microbiota and intestinal SC niche
    - Gut microbiota may be the third factor that affects SC function in the crypts
- Effects of mono-bacterial colonization on intestinal epithelial proliferation
    - Control
        - No microbiota in the gut
    - Mono-colonized
        - Fed individual types of bacteria
        - Ie. A.modestus is the only type of bacteria that is found in that gut
            - It is mono colonized by a.modestus
    - Germ free mouse is a negative control
    - The overall hypothesis is :
        - To see if microbiota affect stem cells and also which ones
            - That is why they mono colonized the gut
    - B.fragilis is used as a negative control
        - Bc you expect no results because it is a bacteria that is not usually found in the gut
    - A positive control
        - This is when you expect to see a positive effect
    - There are 2 negative controls bc the more controls you have the better your experiment

- LPS -dependent changes on crypt homeostasis
    - They made orgainoids and cultured them with LPS of different strains of bacteria