

Assignment 11: Stem Cells

Cell and Tissue Engineering

Problems

1. Please identify which stem cell type or types (embryonic, somatic, or iPS) fit each of the following descriptions. Some have 1 answer, some have more than 1 answer.
 - a. Derivation requires informed consent
embryonic
 - b. Has forced expression of several transcription factors
iPS
 - c. Includes mesenchymal stem cells
somatic
 - d. Totipotent
embryonic
 - e. Low efficiency in creation
iPS
 - f. Limited quantities in the body
somatic
 - g. Used in the Advanced Cell Technology clinical trial
embryonic, somatic, iPS
 - h. Self renews and differentiates
embryonic, somatic, iPS
2. Understanding the three models of stem cell proliferation please discuss which model a tissue engineer would hope is correct and why?

Clonal succession model: in this model, stem cells are in a dormant state waiting to be activated and once triggered any of these stem cells could differentiate and proliferate into a large population of mature cells. These stem cells are available for the lifetime of the organism and have a limited lifespan. The mature clone eventually burns out and a new stem-cell clone take over for cell production.

Deterministic model: this model assumes that the stem cells can self-renew and differentiate into a mature cell and a stem-cell daughter. The probability of

self-renewal may not be exactly 50% depending on tissue environment and may be subject to telomere length.

Stochastic model: the behavior of the outcome of differentiation is random in nature; i.e., a stem cell can generate 0, 1 or 2 stem cells as daughter cells; and can be regulated like the deterministic model by factors external to the dividing cell.

A tissue engineer, would like to rely on the deterministic model, which, is based on the notion that stem cells exhibit a deterministic behavior given their response to differentiation stimuli and generate mature cells for tissue engineering and stem cells for self-renewal and population growth.

3. As we saw this week in lecture, there are a limited number of clinical trials using embryonic stem cells. One company, Geron, which pioneered clinical use of hESCs stopped their trial. Begin by reading the article from ScienceMag about Geron. Please explain why Geron halted their clinical trial utilizing hESC-derived oligodendrocytes to treat spinal cord injuries? And second, explain why they stopped pursuits of stem cell research entirely? Does this surprise you?

After a year, Geron decided to stop a trial to treat 8 patients with spinal cord injury, injected with hESC-derived oligodendrocytes stem cells. At that time, it has already spent \$170 million from which \$25 million was a loan from the California Institute for Regenerative Medicine, a government funded institution (Lukovic et al.). It is reasonable to assume that the cost would have at least doubled as such study to be approved by the FDA, requires a continuous monitoring of the patients for injury improvements, adverse events, or comorbidities issues for many years. At the same time, Geron probably anticipated that their funding through Regenerative Medicine, will be cut: from 2011 until today (slide 10-11D), there has been much more NIH funding in non-embryonic and iPSC research compared to human embryonic research. In addition, Geron funded Dr Thomson research in 1998. In 2011, 3 years after, Geron did not yet have any FDA approved stem cell therapy. Geron executive committee, probably then, realized that the amount of investment needed to continue the trial but also their stem cell research; will be too important, and could jeopardize; maybe; other more promising research. With this context; it seems expected that Geron; as a public company under the pressure of investors, took the only decision they could have financially made and decided to stop the trial and pursuing stem cell research altogether.

4. The following review article discusses how chromatic regulation and structure is involved in stem cell creation (iPSCs), pluripotency and differentiation. At the beginning of this semester, we started a discussion on epigenetics - how chromatin compaction can regulate protein expression. In reading this article, you will continue that discussion. After reading, please provide a critical review of no more than 400 words.

This review should include the following points:

- how histone acetylation and methylation regulate gene expression
- the differences in chromatin structure between stem and differentiated cells
- the model of nuclear compartmentalization

Article: Serrano, L., Vazquez, B.D., and Tischfield, J. Chromatin Structure, pluripotency and differentiation. *Experimental Biology and Medicine*. 238: 259-270. 2013.

Acetylation or methylation of lysine residues of histones, are among histones post-translational modifications causing chromatin conformational changes; which then regulate the recruitment of transcriptional factors and other chromatin binding proteins to DNA. Histone acetylation is modulated by two sets of enzymes HATs and HDACs, both involved in the regulation of gene transcriptional programs. Inhibition of HDACS promote cell proliferation and reprogramming. Class I, II, III HDACs including Sirtuins play a role in limiting the reprogramming process induced by the transcription factors Nanog, Sox, Oct4; and HDAC inhibitors such as VPA or TSA significantly enhance differentiation by down-regulating pluripotency genes. Histone methylation marks of histone H3 are often involved in regulation of gene expression (H3K4me1), transcriptional elongation (H3K36me3) and gene silencing (H3K9me3, H3K27me3). In ESC, bi-valent domains are defined by the simultaneous presence of both H3K27me3 and H3K4me3 marks; genes which have only H3K27me3 or none of these marks are not involved in cell differentiation; and genes involved in cell differentiation present only H3K4me3 mark, and become active. In ESC and iPSC; H3, H4 acetylations; and H4K36me2, H3K4me3 levels are increased whereas heterochromatin is reduced which correlates to a more open chromatin organization important for pluripotency. Chromosomes in the nucleus, are organized in ~1Mb non-overlapping territories (CT) of open and closed chromatin domains. Pluripotency genes activation correlates with gene

positioning within CTs. Chromatin decondensation and chromatin looping out of the CT increase this transcriptional activation, and also, enable long-range regulatory gene interactions. Nuclear domains act as barrier against the spreading of heterochromatin and partition chromatin into transcriptionally active and inactive chromatin regions, and include:

- **Replication domains:** large chromosome domains where replication timing is regulated. Upon differentiation of ESCs to NPCs, chromatin domains reorganize switching from early to late and late to early replication timing. Concentration of active histone marks is found at domain boundaries: making these domains part of the pluripotency signature.
- **Lamina-associated-domain (LADs) and LOCKS domains:** in differentiated cells, LOCKs overlap with LADs, and presence of inactive chromatin domains are important in silencing non-lineage-specific genes by facilitating their association with the nuclear lamina. Both types of domains are enriched in CTCF binding sites. Contrary to LADs, LOCK distribution and abundance; change upon differentiation.
- **Topological domains:** are local chromatin interaction domains enriched with SINE elements, housekeeping and tRNA genes.

Upon cellular reprogramming, these different types of nuclear domains revert to the undifferentiated state to the exception of early to late replicating domains.

Sources:

- Geron Bails Out of Stem Cells: <https://www-science-org.proxy1.library.jhu.edu/content/article/geron-bails-out-stem-cells>

- Lukovic, Dunja, et al. "Perspectives and Future Directions of Human Pluripotent Stem Cell-Based Therapies: Lessons from Geron's Clinical Trial for Spinal Cord Injury." *Stem Cells and Development*, vol. 23, no. 1, Jan. 2014, pp. 1–4. *DOI.org* (*Crossref*), <https://doi.org/10.1089/scd.2013.0266>.
- Serrano, Lourdes, et al. "Chromatin Structure, Pluripotency and Differentiation." *Experimental Biology and Medicine*, vol. 238, no. 3, Mar. 2013, pp. 259–70. *DOI.org* (*Crossref*), <https://doi.org/10.1177/1535370213480718>.

Rubric

Question	Component	Total Point Value
1	A	1.5
	B	1.5
	C	1.5
	D	1.5
	E	1.5
	F	1.5
	G	1.5
	H	1.5
2		5
3		5
4		8
Total		30