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Collagen XIα1 and the Stem Cell Theory of Cancer

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Collagen XIα1 and the Stem Cell Theory of Cancer

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Abstract: Cancer stem cell (CSC) theory hypothesizes that heterogeneity within tumors is not a mere consequence of random mutation and clonal evolution, but results from an intrinsic hierarchy of cells. The KeWe cell line was isolated and characterized in Dr. Oxford's lab. Characterization has included the determination of conditions for maintenance in cell culture for extended periods of time and using different techniques to count the cells to characterize cellular proliferation rates. Collagen XIα1 can be found throughout the body in a variety of places including tendons, skin, ligaments, interstitial tissue, dentin, blood vessels, the cornea, intervertebral discs, muscle, bone, and cartilage. The purpose of this research is to examine the proliferation rates of the KeWe cell line and analyze them to see if they meet the criteria of stem cells and would therefore provide a model system for the investigation of the stem cell theory of cancer. In order to fulfill this research, confirming the high proliferation rate in the cells and identifying the signaling pathways that are active will be the first steps. After confirming the stem cell nature of the KeWe cell line, we propose to use Collagen XIα1 to control stem cell-like behaviors that are important in cancer initiation and progression. Changes in gene and protein expression will be analyzed using high throughput qPCR and mass spectrometry. Collagens are the most abundant protein in the body, and changes in the Collagen XIα1 expression have been identified in cancers and may play a role in disease progression.

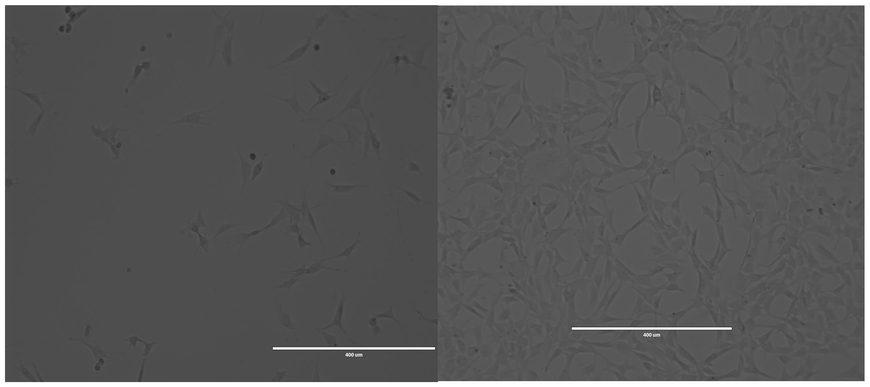

Figure 1: KeWe Cell Line Day 1 & 21

BACKGROUND:

- **KeWe Cell Line:** Recently isolated from zebrafish cell line that is believed to have stem cell-like characteristics. Previously, they have also been shown to have mesenchymal stem-cell markers, such as Vimentin, WNT3A, C-MYC, and SOCS3A. Additionally, the protein Proliferating Cell Nuclear Antigen (PCNA) was highly expressed in the KeWe cell line.
- **Relevance:** We propose that if the KeWe cell line has stem cell-like tendencies, we can use it in combination with Collagen XIα1, as we know that changes in the expression of Collagen XIα1 have been characterized in cancers and potentially play a role in the progression of cancer.
- **Future Direction:** Only the initial steps have been taken thus far in the experiment and we are still gathering data to confirm the optimal proliferation rates based on the different types of media used in the lab. Once we confirm that the KeWe cell line has a high proliferation rate, we intend to use Collagen XIα1 to control stem cell-like behaviors and analyze gene and protein expression through qPCR and mass spectrometry.

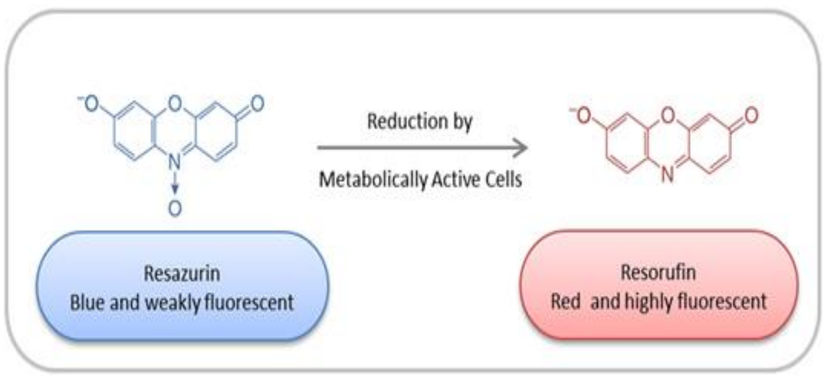

Figure 2: Process of how alamarBlue® works in the media and cells.[1]

alamarBlue® Cell Viability Assay:

•alamarBlue® cell viability reagent functions as a cell health indicator by using the reducing power of living cells to quantitatively measure the proliferation of various human and animal cell lines, bacteria, plant, and fungi allowing you to establish relative cytotoxicity of agents within various chemical classes.[2]

•Advantages to using alamarBlue® include: providing accurate measurements, high sensitivity, no cell lysing, and safe to use.[3]

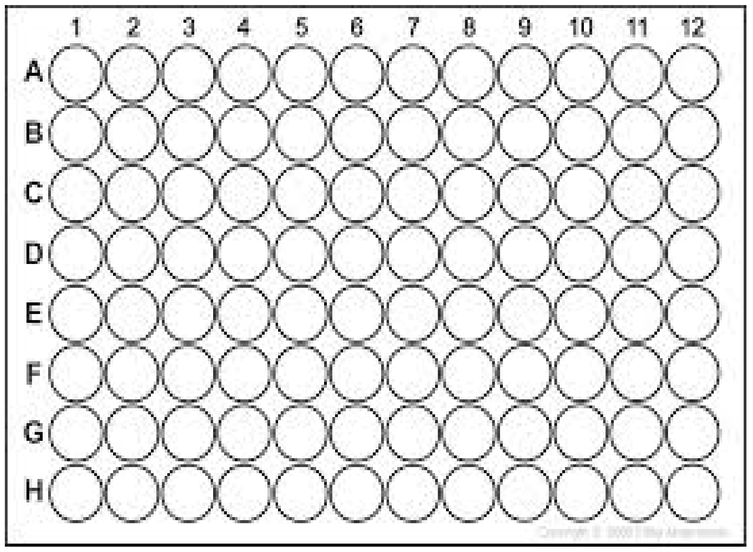

Figure 3: 96-Well Plate Media Order A-G: DMEM, DMEM w/o FBS, DMEM F12, RPMI, Chondrogenic, Adipogenic, and Osteogenic. 96-Well Plate Cell Count Order 1-3: 500 cells; 4-6: 1500 cells; 7: Control.

RESEARCH STRATEGY:

- We picked different medias to find which media would provide us with the highest proliferation rates to use in further experiments.
- Also, we wanted to see if the media would provided different lineages so we could use in future experiments dealing with Collagen XIα1.

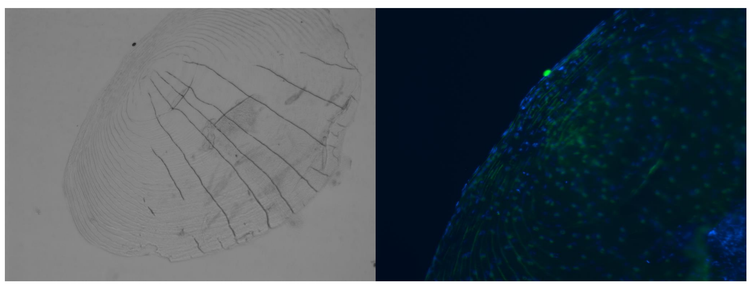

Figure 4: Elasmoid scale from zebrafish. (left) Anterior region consists of concentric circles. (right) DAPI stain and GFP markers reveal cells of interest.

MATERIALS AND METHODS:

- Plate either 500 or 1500 cells into designated wells in a 96-well plate in DMEM media
- After 4 hours, aspirate DMEM and replace with the different types of media listed prior. Add the alamarBlue® into each well.
- Every 2 hours, starting at 0 hour, take a reading in the 96-well plate reader and record the fluorescence at (540,590) with a 70 sensitivity
- Continue taking readings up to 100 hours, reading sporadically after the 24 hour mark.

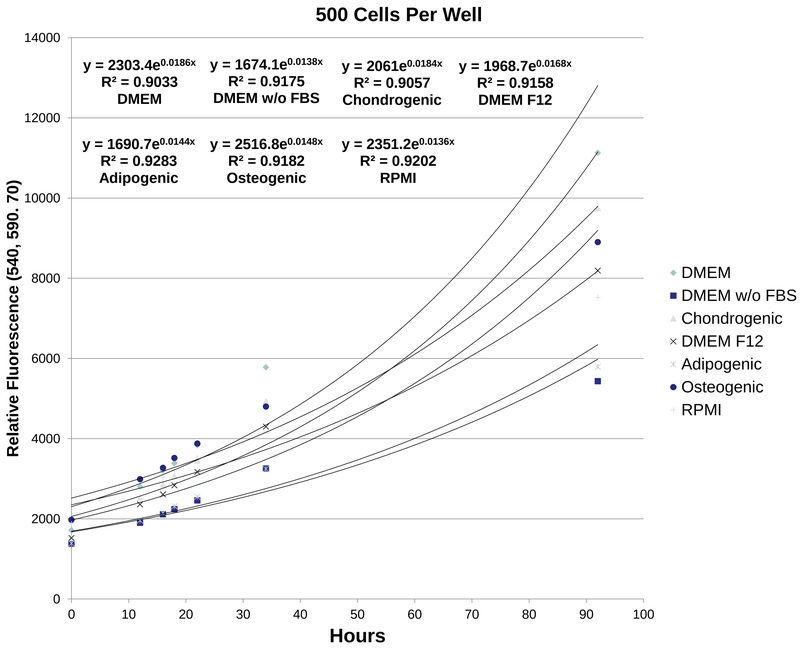

Figure 5: Graphed Results from alamarBlue® Cell Proliferation Assay

CONCLUSION:

- According to our alamarBlue® Cell Proliferation Assay, our data shows that the KeWe cell line proliferation rates are exponential in respect to time.
- This data can be used to help determine the best types of cell culture media to use for the highest proliferation rates of the KeWe cell line.

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References:

1) "The Fast, Simple and Reliable Reagent to Assess Cell Health." *AbD Serotec.* Bio-Rad Laboratories, n.d. Web. 13 Apr 2014.
2) "alamarBlue® Cell Viability Assay Protocol." *Life Technologies.* Thermo Fisher Scientific Inc, 2014. Web. 12 Apr 2014.
3) "alamarBlue® - Rapid & Accurate Cell Health Indicator." *Life Technologies.* Thermo Fisher Scientific Inc, 2014. Web. 12 Apr 2014.