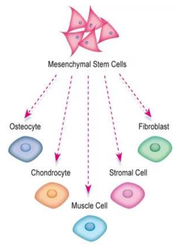
Evaluating the Potential of CD264 as an Effective Biomarker for Cellular Aging in Mesenchymal Stem Cells

Amaris Lewis

Introduction

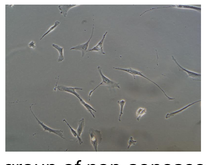
Human mesenchymal stem cells (hMSCs) are non-specialized, multipotent stromal cells with the regenerative potential to differentiate into one of various types of tissue cells. hMSCs offer the unique ability to replace damaged cells in the body, thus providing a therapeutic capability of treating disease.



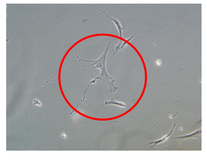
Stem cells present a high degree of clonal heterogeneity, making it difficult to identify which cells are capable of repopulating tissues or are more prone to undergoing senescence. Cell senesence is the point at which cells cease to divide and may be caused by DNA damage or stress factors.

Purpose

The purpose of this research is to find a cell surface biomarker of cellular aging so that optimal cell cultures may be identified despite genotypic variation. If a biomarker for cellular aging in MSCs is identified, then the presence of this biomarker may be used to distinguish advantageous cell cultures from those more likely to becoming senescent, which will aid in selecting ideal cultures to be used in stem cell therapies. CD264 is a cell-surface decoy receptor that inhibts the formation of a death-inducing signaling complex.



A group of non-senescent stem cells



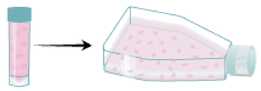
A senescent stem cell (in center)

Hypothesis

If stem cells have a relatively low presence of CD264, then they will demonstrate relatively high colony-forming efficieny. CD264 is a cell-surface decoy receptor that inhibts the formation of a death-inducing signaling complex. Based on further knowledge that senescent tumor cells demonstrate significant cell surface CD264 expression, CD264 likely reveals cell age and proliferative potential.

Methods

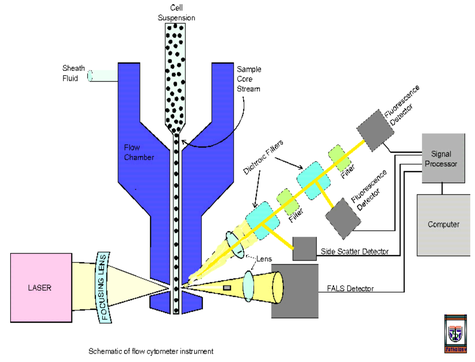
Growing MSC Cultures



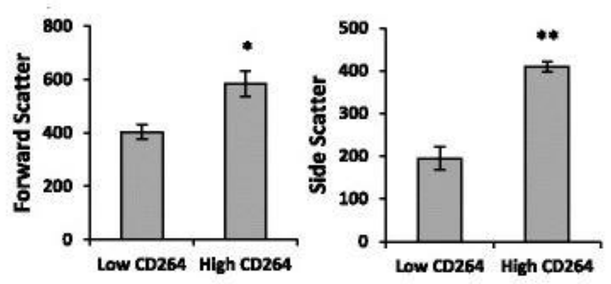
MSCs were harvested from bone marrow of adult volunteers. Cultures were inoculated at 100 cells/cm^2 on plastic T-flasks for amplification. Complete culture media with antibiotics (CCMA) included α-MEM, 2mM L-glutamine, 17% fetal bovine serum, 100 U/mL penicillin, and 100 μg/ml streptomycin. Cell cultures were maintained in a humidified incubator at 37℃ and 5% CO_2.

Flow cytometry

MSCs were detached from the plastic surface of T-flasks via trypsinization. Then, immunolabeling was performed on the surface of live MSCs to detect CD264 using the marker-specific fluorophore FITC. Cells were passed individually through the laser beam to detect fluorescence emitted by the fluorophore. This data was then translated to a series of histograms that permitted the analysis of individual populations. The machine sorted the top 20%, bottom 20%, and parent population into different tubes of ~50,000 cells each.



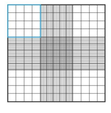
Schematic of flow cytometer instrument



Cell counting

MSCs were thawed, enriched with media, and centrifuged. Cell pellets were dislodged and stained with trypan blue to account for viability. Twenty microliters were transferred from the tube to a hemocytometer and counted visually with a light microscope. Using the calculated concentration, cells were plated onto 3 single-well plates (labeled as CD264+, CD264-, or parent) such that there were approximately 100 live MSCs/dish. Fourteen milliliters of CCMA were added to each plate and cells were maintained in the incubator for 21 days.

$$\text{Total cells/mL} = \text{Total cells counted} \times \frac{Dilution\ Factor}{Number\ of\ squares\ counted} \times 10{,}000$$

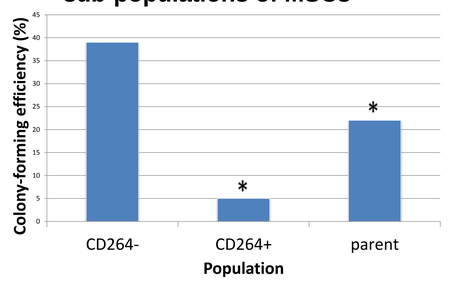


Determining colony-forming efficiency

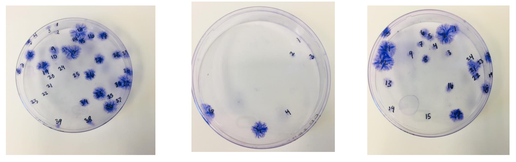
MSCs were inoculated at 100 cells per culture dish with 14 mL CCMA. After two weeks in the incubator, MSCs were stained with crystal violet and colony-forming units (CFUs) were visually counted. Colony-forming efficiency was calculated by dividing the number of colonies by total number of cells plated in the dish (100+/- 10).

Results

MSC Population	Colony-forming efficiency
CD264-	39
CD264+	5
parent	22



* Unpaired t-test statistical value of 0.0020. By conventional criteria, this difference is considered to be very statistically significant.



Populations of CD264-, CD264+, and parent, respectively

Discussion

The results suggest that the presence of CD264 has a negative correlation with cellular aging in MSCs. The population of CD264- cells (lowest 15%) displayed the highest colony-forming efficiency at 39% while CD264+ cells (top 15%) demonstrated a colony-forming efficiency of 5%. The statistical t-test value between parent and CD264+ populations of 0.0200 suggests that this variable is a highly effective biomarker for detecting a cell's affinity to undergoing senesence relative to other samples.

Conclusion

Overall, the experiment agreed with the initial hypothesis that colony-forming efficiency would decrease as the presence of CD264 increased. Hence, CD264 may emerge as an effective biomarker for identifying stem cell cultures to be used in therapeutic stem cell technologies. Potential error lies in the fact that only one trial was completed for the population of CD264+ cells. It is still unknown why CD264 has a negative correlation with cell proliferation, so future research may include investigating cell signaling pathways that produce this effect.