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Skin to Blood

Background, Purpose and Hypothesis

Waiting times and rejection rates for allogenic bone marrow transplants are currently pressing issues in leukemia treatment[1]. Many die while waiting for a bone marrow, and others suffer from immune rejection after transplants. Autologous transplants, on the other hand, reduce the risk of rejection, but substantially increase the risk of relapse[2]. In order to circumvent such problems, we designed a project that explored the possibility of creating bone marrow from the patient's own skin stem cells. Present in the epithelial layer of every adult, these pluripotent cells have been shown to differentiate into cell lineages unrelated to skin including neurons[3].. However, they have not yet been shown to differentiate into the blood cell types found in the bone marrow. Thus, the purpose of the experiment was to explore the possibility of differentiating skin-derived stem cells into hematopoietic progenitors. Because of strict ethical regulations regarding use of human cells, experiments were performed on mouse-derived stem cells as a human model. We hypothesized that the skin-derived stem cells would differentiate into hematopoietic progenitors which express blood-related surface markers CD27 and CD34 when cultured in media containing bone marrow growth factors.

Procedure

Murine bone marrow was isolated by mentor according to standard protocol [4] and cultured at 37°C in two different media (see appendix A for media compositions). At regular intervals of 24 hours, the cultures were centrifuged and the supernatant was siphoned off filtered and stored at

4°C. The cell pellets at the bottom of the tubes were re-suspended in media and incubated at 37°C for another 24 hours. This process was repeated for 7 days.

Skin stem cells (salvaged from mentor's own experiments) derived from fetal mice were cultured in the harvested supernatant at 38.5°C in 24 wells for three weeks. Every three days, the medium was changed. Cells from six of the wells were fixed in paraformaldehyde on each of days 10, 15, 20 and stored at 4°C. Two fixed wells from each of the three days were used for immunocytochemistry. Immunocytochemistry was performed in accordance with standard protocol [5]. The immunocytochemistry procedure was also performed on a sample of untreated stem cells to serve as the control.

Further, two more fixed wells from each of the three days were used for Hematoxylin and Eosin staining following standard protocol [6].

All aspects of the experiment were repeated to confirm preliminary results.

Results/Observations

In the first replication, samples cultured in medium A supernatant and fixed on 15 exhibited significantly more fluorescence representing CD34 and CD27 expression than that of the control. Since both CD34 and CD27 are expressed in blood cells, we expected the red CD34 antibody and green CD27 antibody fluorescence to be co-localized. Indeed, this was observed to be the case. Such results confirm that the signals observed were not due to random background fluorescence, but are in fact representative of the surface proteins found on the cells. The sample fixed on day 20 exhibited no significant red or green fluorescence. This may be due to the unhealthy condition of cells, which were noted to be apoptotic from the DapI nuclear stains. In the second replication, CD27 and CD34 expression were again highest in the samples fixed on

day 15. Low levels of CD27 and CD34 expression were observed in samples fixed on day 10 for both replications. This suggests that skin stem cells cultured in medium A supernatant do not undergo significant hematopoietic differentiation before day 10 of culture.

Samples cultured in medium B supernatant exhibited a significant level of CD27 and CD34 expression on days 10, 15, and 20 in both replications. Again, fluorescence for both markers appeared to be co-localized. These results show that the skin-derived stem cells successfully differentiated into hematopoietic lineages. See Appendix B for a graphical representation of marker expression.

Fixed cells stained with Hematoxylin and Eosin revealed structures characteristic of various blood cell types, including pink cytoplasm (specific to red blood cells), salmon pink cytoplasmic staining with granules (specific to neutrophils), bi-lobed nuclei (specific to eosinophils). Because interpretation of stain results can be subjective, however, RT-PCR analysis will be used to conclusively determine which hematopoietic lineages are present in culture.

In conclusion, murine skin-derived stem cells differentiated into blood cells and blood progenitors when cultured in media containing bone marrow cytokines. Since hematopoietic progenitors are the most vital components of the bone marrow, this discovery can lead to a novel, easily accessible source of bone marrow that can be grown from a patient's own skin stem cells.

Ongoing Research

More analysis will be conducted on the cells to confirm their differentiation into blood lineages. The cells' RNA will be tested using RT-PCR, a method of amplifying the specific sequences of interest. Gel electrophoresis will be performed to determine the presence of the particular mRNA transcripts. Specifically, we will be looking for sequences that pertain to blood, such as

macrophage scavenger receptor, CD34 surface marker, and other particular genes. The results of RT-PCR will attest more conclusively to the hypothesis that the skin stem cells differentiated into blood cell lineages.

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References

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APPENDIX A

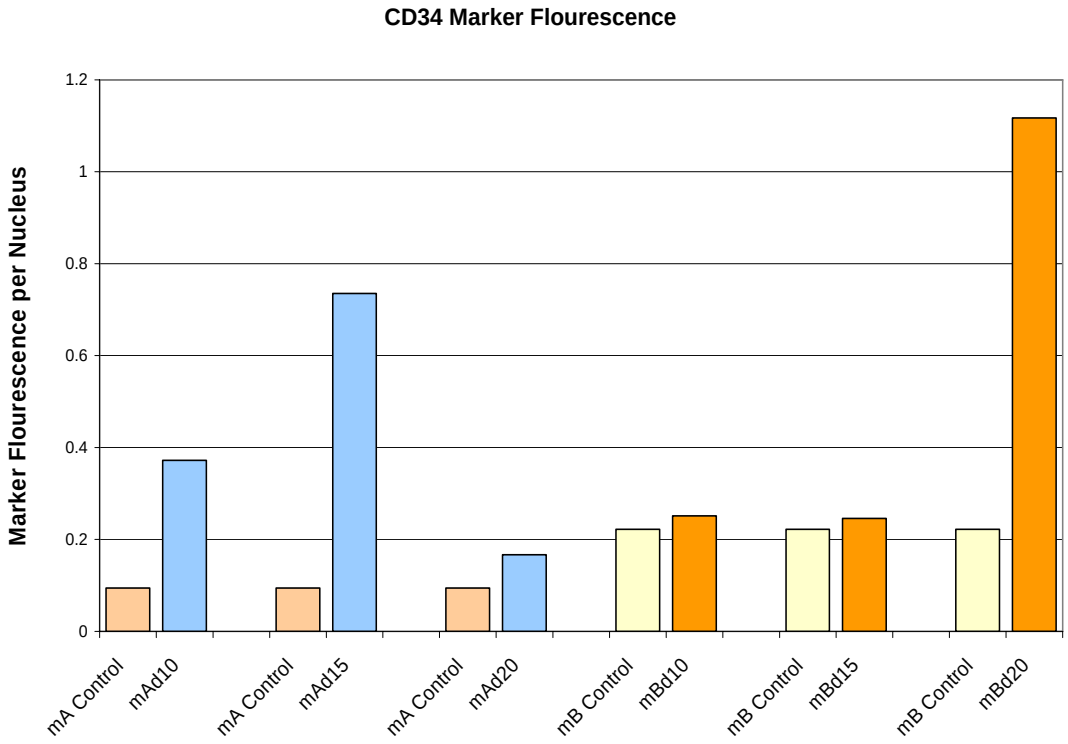
Composition of Medium A:

10% FBS made up with DMEM

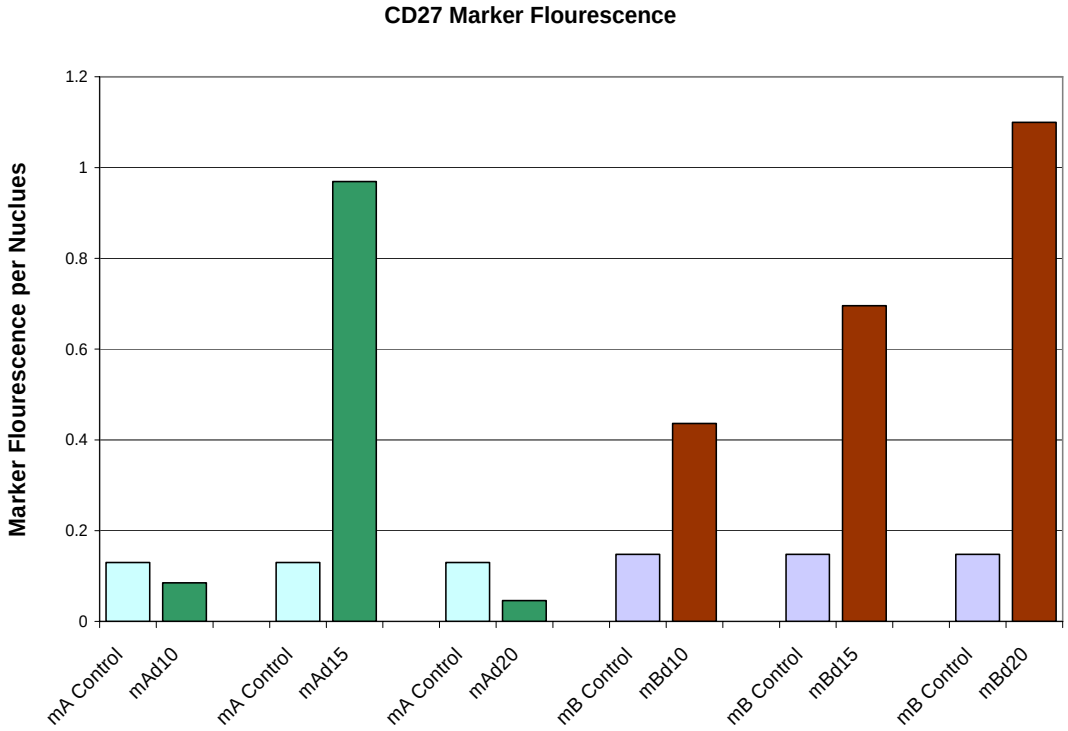
Composition of Medium B:

20% FBS, 2nM L-glutamine, 0.1nM B mercanptoethanol, 1% non-essential amino acids, made up with DMEM

APPENDIX B



Legend
m- media in which cells were cultured
d- day cells were fixed



Legend
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