Exploring the role of glycolysis in mouse stem cell differentiation

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Introduction

Normal stem cells are proliferative, undifferentiated cells that have the ability to differentiate into different types of cells in the body.

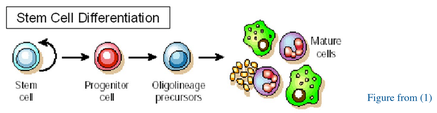
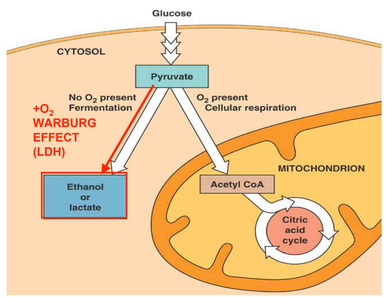


Figure from (1)

Previous studies on cancer cells suggest that there is a small tumor-initiating cell population which can give rise to the entire tumor mass; these cells are known as cancer stem cells (1). They are thought to initiate tumorigenesis and are the putative cause for disease recurrence and metastasis. Therapeutic targeting of these cells is therefore important for cancer treatment.

Cancer cells, like normal stem cells, are highly proliferative. Previous work suggests that both cancer stem cells and normal stem cells may share a common metabolism, one in which the natural pathway for glucose breakdown, glycolysis, is shunted away from the mitochondria, and pushed toward lactate production. This phenomenon is known as the Warburg effect (2).



Less differentiated cells (such as stem cells) are proliferative and produce lactate, while differentiated cells are non-proliferative and depend less on fermentation. Our hypothesis is that normal and cancer stem cells may be induced to differentiate by modulating their shared metabolic dependency.

Our goal has been to understand how normal stem cells and cancer stem cells can be induced to differentiate by modulating the glycolytic pathway. Our studies hope to elucidate a novel mechanism for differentiation therapy of cancer stem cells and may have further implications in our understanding of normal stem cell biology.

My Specific Objective: To characterize the effects of chemical modulation of glycolysis on differentiation by using chemical inhibitors of glycolysis and measuring the effects on C2C12 mouse myoblast (a precursor cell line) differentiation.

Materials and Methods

- For our experiments we used two different cell lines:
  - C2C12 mouse myoblast cells: Myoblast cells are adult precursor stem cells. These cells fuse to form mature muscle fibers when differentiated. We added compounds (chemical inhibitors of glycolysis) to the myoblast cells, and measured effects on differentiation. This is a robust model system for studying differentiation, since muscle cell differentiation can be induced and scored with high reproducibility.
  - Mouse embryonic stem cells (ES cells): Mouse ES cells are undifferentiated pluripotent cells that can differentiate into any type of cell in the body. We used the mouse CCE stem cell line for our assays.
- Mouse ES cells were passaged every other day and mouse C2C12 cells were passaged every 3-4 days upon reaching confluency.
- Compounds used to inhibit glycolysis were:
  - Specific enzyme inhibitors of lactate dehydrogenase (the enzyme which is responsible for the conversion of pyruvate to lactate):
    - Oxamic Acid
    - Silver Nitrate
  - Compounds (found from a screen performed in the Schreiber lab) that inhibit lactate production:
    - Crinamine
    - Lycorine
    - Tomatine
    - 5HT
    - DOI
    - 6AN

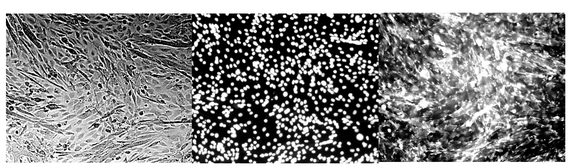
    The mechanism by which these compounds work is unknown.

Assay Development: Mouse Myoblast Cells

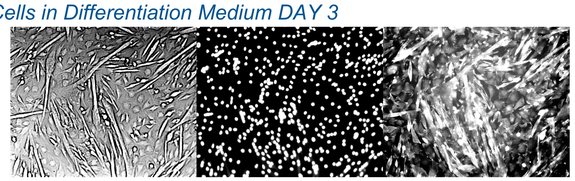
We found that on day 3 of culture C2C12 cells differentiated without significant background noise, i.e. above the level of spontaneous differentiation which occurs despite non-differentiating conditions.

Control: Cells in Non-differentiated Medium DAY 3

Phase Contrast (cell outlines) | Hoechst (nuclear staining) | Phalloidin (cytoskeleton staining)

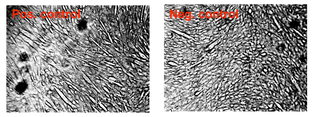


Cells in Differentiation Medium DAY 3

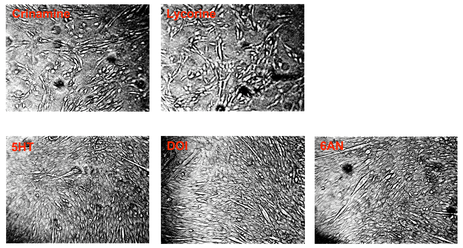


Results: Mouse Myoblast Cells

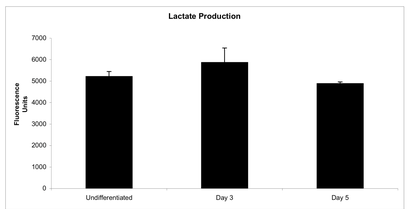
We tested 8 inhibitors of lactate production on our mouse myoblast cells to see if they induced differentiation. Differentiation was measured by myotube formation as determined by microscopy. The positive control was grown in 2% horse serum, and the negative control was grown in 10% horse serum.



Of the 8 compounds tested, the five below appeared to have effects on either cell viability or differentiation.



Measurement of Lactate Production: Media was changed 24 hours prior to assaying for lactate production. Supernatant was collected the following day. Lactate production was measured by fluorescence, with three different conditions:Undifferentiated cells, differentiated cells day 3 of culture and differentiated cells day 5 of culture.



Conclusions

Compounds: From our preliminary studies on metabolic inhibitors, we see that at least two of the compounds (crinamine and lycorine) primarily affect cell viability; this is apparent in reduced cell number as compared to control cells. Three of the eight compounds (5HT, DOI, and 6AN) have modest effects on differentiation. We are currently repeating these experiments to verify preliminary results, and we will quantify differentiation by immunofluorescence staining against myosin heavy chain.

Lactate assay: From our studies on lactate production in differentiating C2C12 cells, it appears that there is an initial increase in lactate production (day 3) which tapers as the cells differentiate (day 5). This is likely to correlate with cell proliferation which we also believe initially increases before cells terminally differentiate and exit the cell cycle.

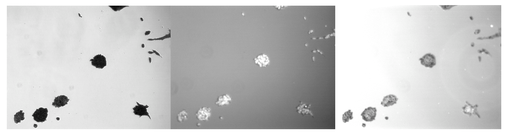
Future Directions

In the future we hope to apply this system to embryonic stem cells, which we have begun to explore. Using tissue culture methods and removal of LIF, we tried to induce differentiation in mouse embryonic stem cells (CCE cell line). We tested for differentiation using alkaline-phosphatase and oct4 antibody staining. We are still working on assay development with this system. We have not been able to differentiate these cells, as evidenced by continued expression of alkaline phosphatase and oct4.

Cells in Differentiation Medium DAY 1

Alkaline-phosphatase (stem cell marker) | *Hoechst (nuclear staining)* | *OCT4 (stem cell marker)*

Cells in Differentiation Medium DAY 3



In the future, we intend to establish a genetic approach for modulating glycolysis. RNA interference tools (RNAi) will be used to screen for target metabolic genes which may induce differentiation.

Literature cited

1. Reya, Tannishtha, Sean K. Morrison, Michael F. Clarke, Irving L. Weissman. "Stem cells, cancer, and cancer stem cells." Nature 414(2001): 105-111.
2. Warburg, Otto. "On the Origin of Cancer Cells." Science 123(1956): 309-314.

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