Effect of mTOR on Hematopoietic Differentiation of hESC

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INTRODUCTION

In adult mammals, most hematopoietic cells are derived from self-renewing hematopoietic stem cells that reside in the bone marrow.[1] These cells then differentiate into multipotent progenitor cells, which are not self-renewing, which then differentiate into either common lymphoid progenitor cells or common myeloid progenitor cells.[1] These cells then differentiate further, and eventually into mature cells.

mTOR is an evolutionarily conserved checkpoint protein kinase that is involved in many cellular functions, including cell growth and proliferation[2]. Changes in mTOR, including inhibition, have been shown to effect multiple types of hematopoietic cells, including macrophages, T-cells, B-cells, and myeloid cells[3]. Based on previous data showing that mTOR affected hematopoietic cells, we chose to study the effect of mTOR on hematopoietic differentiation.

AIM

The goal of this study was to investigate the effects of mTOR activator compound on hematopoietic development from ESC in vitro.

METHODS

We differentiated hESC into hematopoietic lineages using the protocol below. We used mTOR-reporter hESC line (H1-TOSI) and added mTOR activator (MHY) on day 9 of differentiation. The effects of mTOR were evaluated using qPCR, FACS, and colony forming assays. In addition, the change in mTOR expression over differentiation was also evaluated using FACS on days 4, 6, 8, and 10.

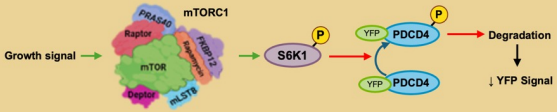

Figure 1: Venus probe pathway

Essential 8	Essential 6	Stem Span
VEGF		
CHIR99021	SCF	
BMP4	SB431542	IL-3, TPO, Flt-3, IL-6, IL- 11, IGF-1, EPO
	bFGF	

Table 1: Protocol for differentiation into hematopoietic lineages

RESULTS

We first examined gene expression profile data using qPCR (figure 2). In this experiment, we found that MHY had a tendency to increase hematopoietic gene expression, but it was not always to a significant level. Data from related experiments also showed variable results.

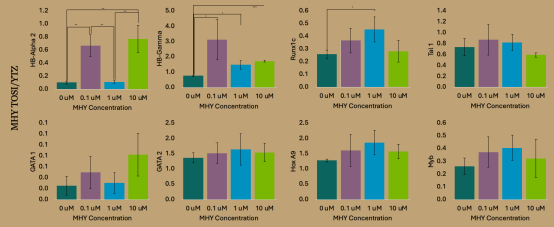

Figure 2: qPCR data for the MHY-treated cells with the mTOR probe.

We also examined the colony forming assay from HPCs (figure 3) We found that there were very few mixed colonies with granulocytes, erythrocytes, macrophages, and megakaryocytes. Many of the colonies were macrophage. While there was some variance with different dosages, no patterns were observed. As in our qPCR analysis, data from related experiments showed variable results.

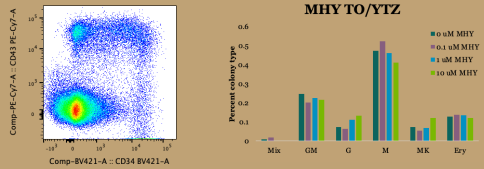

Figure 3: Colony-forming unit analysis data for the MHY-treated cells with the mTOR probe and representative sample of flow cytometry data used to sort.

During the mTOR expression over time analysis, and interesting result was seen. On day 4, two distinct Venus-positive (and therefore low in mTOR) populations can be seen (figure 4). Over time, the group with the lower Venus expression appears to fade away and is completely gone by day 10.

RESULTS

Figure 4: Venus expression over time in H1 TOSI/YTZ ESCs.

CONCLUSION

- mTOR signaling is activated during hematopoietic cell production from human embryonic stem cells.
- mTOR agonist and mTOR antagonist showed some effects on hematopoiesis with variations among cell lines, but these effects require further investigation into culture optimization and mTOR compound dosage.
- The two distinct Venus-positive populations seen in the surface marker analysis represents two populations with differing low-mTOR activity that may differentiate differently.

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