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TP 114

Media Component Analysis during Human Primary T Cell Culture using a Triple Quadrupole Mass Spectrometer

Evelyn H. Wang[1]; Stephen Kurzyniec[1]; Erin Strom[2]; Tammy Jones-Lepp[2]; Amanda Leisgang[2]; Andrew Ortiz[2]; Jefferson Kinney[2]; Yoshiyuki Okamura[1]

[1]Shimadzu Scientific Instrument, Columbia, MD; [2]University of Nevada Las Vegas, Las Vegas, NV

1. Overview

Using a rapid and multicomponent LCMS method to analyze T cell culture media during the expansion process to ultimately increase titer

2. Introduction

Chimeric antigen receptor (CAR) T cell gene therapy has shown success in treating cancers such as leukemias and lymphomas. A CART cell therapy consists of T cell extraction, enrichment and activation, transduction, expansion, isolation, and administration steps. The composition of the cell culture media used during the expansion phase can have a significant impact on the final quality of the product CART cells and the therapeutic efficacy. Therefore, obtaining comprehensive real-time media composition results to monitor cell consumption and secretion prior to harvest will be beneficial. The data will provide insight on the progress of the cell growth and allow the researchers to intervene, if modification is necessary, to increase the T cell's and CART cell's quality and quantity.

3. Method

Experimental growing flasks containing primary human T cell (to mimic the CAR T cell expansion process), T cell expansion medium, human CD3/CD28 T cell activator, cytokine, and IL-2 were incubated at 37 °C with 5% CO_2. In this study, commercially available T cell was used to mimic the CAR T cell expansion process. The result obtained from the T cell culture should be applicable to the CAR T cell expansion workflow. In the two experimental growing flasks, flask A did not go through any media exchange throughout the experiment while flask B went through fresh media exchange at day 3, 5, and 7. Media samples were collected every two hours in the first day and every six hours afterwards.

T Cell media samples were subjected to protein crash using acetonitrile and centrifugation at 15,000 rpm for 10 min at 4°C. Supernatants were further diluted with water prior to analysis. (Figure 1) A reversed phase gradient chromatography was done on a Shimadzu Cell Culture Profiling column (150 mm x 2.1 mm, 3 µm). Mobile phases for the chromatography were water and acetonitrile with 0.1% formic acid.

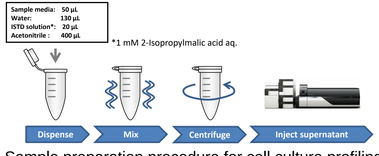

Figure 1. Sample preparation procedure for cell culture profiling analysis

Triple quadrupole mass spectrometry with its fast scan and polarity switching speed is a desirable tool for comprehensive media composition analysis. A Shimadzu LCMS-8060 triple quadrupole mass spectrometer with the capability to simultaneously analyze multiple compound groups was used to analyze T cell culture media. A 17-minute LCMS method that analyzes 144 cell growth related compounds was developed for T cell/CART cell culture media analysis. (Table 1) Cell culture related compounds including amino acids, nucleic acids, metabolites, sugars, and vitamins were simultaneously analyzed by MRM in both ESI positive and negative modes. A total of 40 media samples were collected and analyzed from each T cell culture experimental flask for the whole culture period (9 days). Duplicate results were obtained for each sample.

Table 1. 144 registered compounds in the Cell Culture Media Profiling Method Package version 3.

Amino acids and their metabolites		Nucleic acids and their metabolites
1-Methylhistidine	Glycyl-glutamine	3-Aminoisobutyric acid
2-Aminoadipic acid	Histidine	3-Aminopropanoic acid
2-Aminobutyric acid	Homocysteine	Adenine
3-Hydroxyanthranilic acid	Homocystine	Adenosine
3-Hydroxyisobutyric acid	Hydroxykynurenine	Adenosine monophosphate
3-Methyl-2-oxovaleric acid	Hydroxylysine	Cytidine
3-Methylhistidine	Indole-3-acetic acid	Cytidine 3',5'-cyclic monophosphate
4-Aminobutyric acid	Isoleucine	Cytidine monophosphate
4-Hydroxyphenyllactic acid	Kynurenic acid	Cytosine
4-Hydroxyproline	Kynurenine	Deoxyadenosine
5-Glutamylcysteine	Leucine	Deoxycytidine
5-Hydroxytryptophan	Lysine	Deoxycytidine monophosphate
5'-Methylthioadenosine	Methionine	Deoxyguanosine
5-Oxoproline	Methionine sulfoxide	Deoxyguanosine monophosphate
Acetylcarnitine	N-Acetylaspartic acid	Guanine
Alanine	N-Acetylcysteine	Guanosine
Alanyl-glutamine	Norepinephrine	Guanosine 3',5'-cyclic monophosphate
Anthranilic acid	Ophthalmic acid	Guanosine monophosphate
Arginine	Ornithine	Hypoxanthine
Argininosuccinic acid	Oxidized glutathione	Inosine
Asparagine	Phenylalanine	Inosine monophosphate
Aspartic acid	Pipecolic acid	Orotic acid
Asymmetric dimethylarginine	Pipecolic acid	Thymidine
Carnitine	Proline	Thymidine monophosphate
Citrulline	Putrescine	Thymine
Creatine	Saccharopine	Uracil
Cystathionine	S-Adenosylhomocysteine	Uric acid
Cysteine	Serine	Uridine
Cystine	Serotonin	Uridine monophosphate
Dopa	Symmetric dimethylarginine	Xanthine
Formylkynurenine	Threonine	Xanthosine
Glutamic acid	Tryptophan	Xanthosine monophosphate
Glutamine	Tyrosine	
Glutathione	Urocanic acid	
Glycine	Valine	

Sugars	Others
Gluconic acid	2-Aminoethanol
Hexose (Glucose)	2-ketoglutaric acid
Sucrose	Acetylcholine
Threonic acid	Acotinic acid
Vitamins	Citric acid
Riboflavin	Fumaric acid
Niacinamide	Glyceric acid
Nicotinic acid	Glycolic acid
Pantothenic acid	Glyoxylic acid
4-Pyridoxic acid	Isocitric acid
Pyridoxal	Lactic acid
Pyridoxalphosphate	Malic acid
Pyridoxine	Mevalonic acid
Biotin	NAD
4-Aminobenzoic acid	O-Phosphoethanolamine
Folic acid	Penicillin G
Choline	Pyruvic acid
Ascorbic acid	Resveratrol
Cyanocobalamin	Shikimic acid 3-phosphate
Lipoic acid	Succinic acid
Internal Standard	Taurine
2-Isopropylmalic acid	

4. Results

T cell culture media samples of flask A (without media exchange) and flask B (with fresh media exchange) from day 0 to 9 were collected and analyzed. Chromatograms of the media at hour 0 and hour 210 were compared in both experimental flasks. (Figure 2) Flask B at the end of the experiment obtained ~3 times higher live cell count. Differentiating compounds with significantly higher concentrations in flask B were shown on the volcano plot. (Figure 3) Time course area trends for compounds of interest and potential T cell metabolomic mechanism diagrams are shown in figure 4-6.

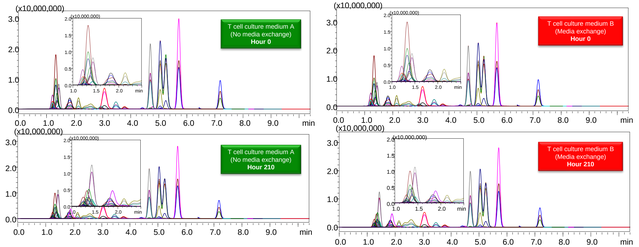

Figure 2. Chromatograms of T cell culture media in flask A (without media exchange) and B (with media exchange) at the beginning (hour 0) and the end (hour 210) of the cell culture period. Differences between the two experimental flasks were especially apparent in early eluting (1 – 2 min) compounds.

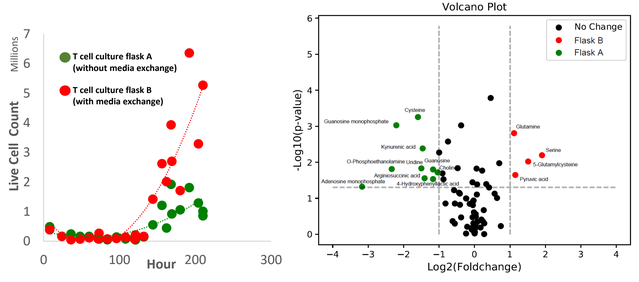

Figure 3. Live T cell count throughout the cell culture period is shown on the left. Flask B with three media exchanges during culture resulted in significant higher live T cell count at the end. The volcano plot of the T cell culture media from flask A and B at the end of the culture period is shown on the right. Differentiating compounds that were significantly higher in flask A and B are shown in green and red, respectively.

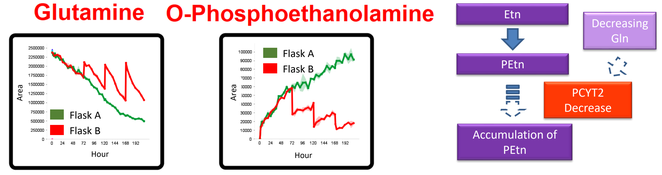

Figure 4. Area trends of glutamine decreased as O-phosphoethanolamine increased during T cell culture. This trend could suggest that glutamine starvation lead to increase in O-phosphoethanolamine by downregulation of the PCYT2 enzyme.[1]

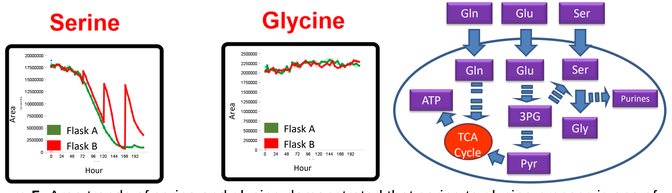

Figure 5. Area trends of serine and glycine demonstrated that serine to glycine process is one of the major factor of upregulate T cell activation. Extracellular serine is required for optimal T cell expansion process as it directly controls T cell proliferative capacity.[2]

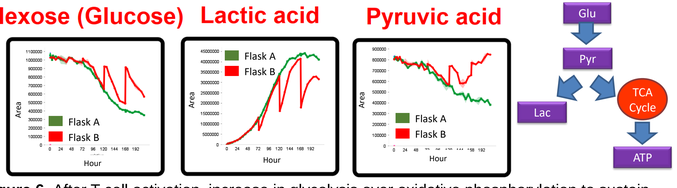

Figure 6. After T cell activation, increase in glycolysis over oxidative phosphorylation to sustain rapid cell growth was observed with the decreasing trend of glucose and the increasing trend of lactic acid.[3]

5. Reference

1. Osawa, Tsuyoshi, et al. "Phosphoethanolamine accumulation protects cancer cells under glutamine starvation through downregulation of PCYT2." Cell Reports 29.1 (2019): 89-103.
2. Ma, Eric H., et al. "Serine is an essential metabolite for effector T cell expansion." Cell metabolism 25.2 (2017): 345-357.
3. Palmer, Clovis S., et al. "Glucose metabolism regulates T cell activation, differentiation, and functions." Frontiers in immunology 6 (2015): 1.