Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Chemical Engineering

Manipulation of metabolism in complex eukaryotic systems to control cellular state

Amy M Su and Mark P Styczynski

The regulation of metabolism is critical to many important cellular processes in higher eukaryotes, and metabolites themselves can have significant regulatory potential in complex phenotypes. Thus, external control of metabolism for both biotechnological and biomedical ends is of great importance. There has been increasing emphasis on using non-genetic approaches for direct, transient control of metabolism and cellular state, with particularly promising applications in stem cell biomanufacturing and cancer. Metabolite-based methods have been used to facilitate expansion of stem cells, to control and prevent their differentiation, and to reprogram cells to a pluripotent state. Similar approaches are also being explored to inhibit the growth of cancer cells.

Address
School of Chemical & Biomolecular Engineering, Georgia Institute of Technology, Atlanta, GA, USA

Corresponding author: Styczynski, Mark P (Mark.Styczynski@chbe.gatech.edu)

Current Opinion in Chemical Engineering 2015, **10**:63–69

This review comes from a themed issue on **Biotechnology and bioprocess engineering**

Edited by **Eleftherios Terry Papoutsakis** and **Nigel J Titchener-Hooker**

For a complete overview see the Issue and the Editorial

Available online 10th September 2015

http://dx.doi.org/10.1016/j.coche.2015.08.004

Introduction

Metabolic control in complex eukaryotic systems has long been an important biotechnological goal. Cell culture engineering was key in enabling efficient, scalable production of antibodies and other proteins via Chinese hamster ovary (CHO) cells grown in bioreactors [1,2] as well other mammalian cell types [3], and improvement of these techniques is still an active research area [4]. For these applications, the endpoint is typically a comparatively simple phenotype: protein secretion. However, as biotechnological applications and the cell culture systems they employ become more diverse and complex, previously developed approaches to controlling mammalian cell metabolism will need to be repurposed and new methods to facilitate fine-tuned control of these systems will need to be developed.

One of the most promising applications of cell culture engineering is the emerging field of stem cell engineering and biomanufacturing [5,6]. Stem cells have significant potential as therapeutics for treating numerous diseases via both autologous and allogeneic approaches. Irrespective of the scientific and biomedical challenges that abound in the use of stem cells as therapeutics, there are at least two practical challenges in translating multipotent stem cells into a viable therapeutic platform: expansion to enable industrial-scale production of cells, and maintaining cells in, or differentiating them to, the appropriate cell type state. Moreover, if one considers the use of induced pluripotent stem cells (iPSCs) to enable autologous transplantation, efficient reprogramming of the cells is another key step that must be optimized. Ultimately, then, a much more complex phenotype must be accounted for in stem cells than in CHO cells: whereas CHO cells are the vehicle for making the desired product (i.e. protein), the stem cells themselves are the desired outcome of the bioprocess, and thus the cell type state (which has manifold possibilities) is the main target to be explicitly monitored and controlled. While some of the same principles used in CHO cell engineering can be applied, there is also increased demand for simple molecular mediators to help direct cells on the complex differentiation landscape, since genetic approaches are often to be avoided for therapeutic applications.

The principles for stem cell biomanufacturing can also be applied to another complex biological system with related, but often opposite, goals: cancer. Rather than encouraging cell growth as in stem cell biomanufacturing, the goal is instead to stop proliferation of cancer cells (in some cases by inducing differentiation [7••]). There are a surprising number of similarities between cancerous cells and stem cells: not only can both cell types create many cells from a few (through proliferation or self-renewal), but they both rely on high glycolytic flux and have a number of uncommon metabolic tendencies [8]. In both cases, there is a preference to avoid genetic interventions for manipulating the cells, whether due to difficulty in delivery, potential genetic side effects, or regulatory approval issues. Lastly, while small molecules have been dominant for decades in cancer treatment, the use of endogenous metabolites (or close derivatives or analogs) is beginning to show great potential, consistent with recent advances in using endogenous small molecules to control stem cells.

Here, we consider stem cells and cancer cells as two important challenges for controlling cell growth and cell

state in complex systems. In both cases, metabolism has recently been shown to be critical in the cells' characteristic phenotypes, and metabolism-based control is emerging as a promising avenue to manipulate cell state and phenotype. We first address the prominent bioprocessing and biotechnological challenge of stem cell biomanufacturing. We then consider similarities between this field and the control of cancer metabolism, with brief discussion of the interesting intersection of these two cell types (cancer stem cells) and what this research area could mean for biomedical applications.

Controlling stem cell state for biomanufacturing

Stem cells are defined by their ability to self-renew (forming identical daughter cells) and differentiate (creating more specialized daughter cells), with many different types and classes of these cells. Embryonic stem cells (ESCs) are derived from preimplantation-stage embryos and are pluripotent, meaning they have the ability to generate any cell type, from any of the three germ layers. (They are not totipotent, though, which entails the additional ability to generate the extraembryonic cell types such as those in the placenta.) As cells differentiate, their range of possible lineages becomes more restricted (they lose potency, from pluripotent to multipotent to unipotent) until they differentiate to a terminal cell type. Multipotent cell types (that can form multiple terminal cell types) such as hematopoietic stem cells (HSCs) or mesenchymal stem cells (MSCs) can be derived from ESC lines or, more commonly, be collected from stem cell populations that persist in adults. Induced pluripotent stem cells (iPSCs) are created from differentiated cells (often fibroblasts of various origin) that are reprogrammed back to a pluripotent state by manipulation of the cells' gene regulatory network. iPSCs are particularly exciting for not only their utility as a source of pluripotent stem cells, but also for the possibility of personalized medicine at a previously unforeseen level of specificity. All of these cell types represent complex phenotypes and cellular states that can be difficult to thoroughly characterize and demonstrate; often the expression of just a few known markers is used as a proxy for differentiation status, though in some cases more detailed functional characterization of cells is also performed.

One of the key practical barriers to stem cell-based therapeutics is the production of enough cells of the correct types for therapeutic doses at a commercial scale. With disease-dependent estimates ranging up to 10^9 or more cells for a single dose [9], multiplied by thousands to millions of patients, a basic two-dimensional adherent culture approach clearly will not scale industrially. Three-dimensional bioreactor formats for culture and differentiation of pluripotent stem cells have sought to eliminate the dependence on tissue culture surface area (an excellent review is available elsewhere [9]). However, the dramatically different physical conditions of bioreactors as compared to adherent culture (introducing elements such as shear stress, degree of mixing, and gas phase composition) lend themselves to numerous challenges in meeting the metabolic needs of the cells. For example, higher cell density leads to greater consumption of nutrients and oxygen, which can be addressed via a well-mixed nutrient and oxygen supply throughout the tank and inside cellular aggregates, and via continuous reactor-level control of these parameters. Higher cell density also leads to greater production of CO_2 that can stay dissolved in the bioreactor and tends to inhibit growth, which must be addressed. Work is ongoing in bioreactor development; one of the most promising recent approaches used a thermoresponsive hydrogel in fully defined conditions for long-term, serial expansion at a high rate (20-fold within 5 days) and for in-reactor differentiation [10••]. As limitations on cell growth and culture are alleviated, even more attention will turn to the establishment of methods for precise control of cell type, particularly because changing culture formats will affect the signals that control differentiation, including endogenous factors such as extracellular matrix and cell-secreted growth factors. Metabolism-based control is a promising candidate to address this issue.

Metabolic phenotypes and differentiation state are linked

There is significant evidence linking the metabolic state of pluripotent stem cells to their differentiation state, suggesting the potential importance of metabolism in differentiation and self-renewal (Figure 1). For example, via a metabolite profiling approach, the oxidation state of lipid profiles was shown to be highly correlated with cell state during embryonic stem cell differentiation [11]. Supplementation with metabolites associated with oxidative metabolism promoted differentiation down neural and cardiac lineages as assayed by protein markers such as βIII-tubulin (neural) or cardiotroponin (cardiac), suggesting that the metabolic changes actually played a functional role. Similar metabolite-focused investigations of induced pluripotent stem cells also showed metabolic changes between induced pluripotent stem cells and their parental cells [12,13] and differences between induced pluripotent and embryonic stem cells. Most recently, it was found that metabolic changes are present even at extremely early stages of differentiation [14•], leading to identification of glycolysis-mediated changes in histone acetylation and metabolite levels that play a role in differentiation. Taken together, these results suggest a central role of metabolism in differentiation and motivate the desire to control metabolism in stem cells.

Endogenous metabolites as soluble differentiation factors

Supporting the hypothesis that metabolism plays a central role in differentiation is that most of the above studies