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Review

Process engineering of stem cell metabolism for large scale expansion and differentiation in bioreactors

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ABSTRACT

Mesenchymal stem cells (MSCs) and pluripotent stem cells (PSCs) emerge as promising tools for tissue engineering, cell therapy, and drug screening. Their potential use in clinical applications requires the efficient production of differentiated cells at large scale. Glucose, amino acid, and oxygen metabolism play a key role in MSC and PSC expansion and differentiation. This review summarizes recent advances in the understanding of stem cell metabolism for reprogramming, self-renewal, and lineage commitment. From the reported data, efficient expansion of stem cells has been found to rely on glycolysis, while during differentiation stem cells shift their metabolic pathway to oxidative phosphorylation. During reprogramming, the reverse metabolic shift from oxidative phosphorylation to glycolysis has been observed. As a consequence, the demands for glucose and oxygen vary upon different phases of stem cell production. Accurate understanding of stem cell metabolism is critical for the rational design of culture parameters such as oxygen tension and feeding regime in bioreactors towards efficient integrated reprogramming, expansion, and differentiation processes at large scale.

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1. Introduction

In recent years, both mesenchymal stem cells (MSCs) and pluripotent stem cells (PSCs) have been widely studied for their potential applications in tissue engineering, cell therapy, disease modeling, and drug screening [1,2]. MSCs, initially derived from bone marrow stroma, display in vitro differentiation potential along various mesodermal lineages including osteoblasts, chondrocytes, and adipocytes [3]. PSCs, including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), have unlimited proliferation capacity and a broad spectrum of differentiation abilities into all cell types from the three-germ layers [4,5]. While ESCs are needed to be derived from the inner cell mass of the blastocyst, it is recently found that somatic cells can be reprogrammed using pluripotent genes or small molecules to become iPSCs, which have similar functional properties to ESCs [5–7].

Stem cells have unique energy and biosynthetic requirements for maintaining the pluripotent state, for induction towards lineage-specific cells, and for reprogramming of somatic cells [8]. In general (except for totipotent stem cells), proliferating stem cells predominantly utilize glucose through aerobic glycolysis (i.e., known as the Warburg effect) rather than oxidative phosphorylation (OXPHOS) [9], while upon differentiation the metabolic pathway shifts from glycolysis to OXPHOS (Fig. 1). Indeed, for fast-growing cells, glycolysis supports a high degree of nutrient incorporation into biomass [9]. Moreover, proliferating cells display an increased lactate production when an excessive level of glucose is provided, a process known as the Crabtree effect [10]. The major by-product of glucose metabolism through OXPHOS and tricarboxylic acid (TCA) cycle is carbon dioxide (CO_2), while the lactate is produced by glycolysis (Fig. 1). The lactate accumulation has been found to display variable degree of toxicity among various stem cell populations which can reduce cell proliferation [11,12]. Therefore, oxygen, glucose, and amino acids, the major components involved in the metabolic pathways, play a crucial role in the regulation of stem cell proliferation and differentiation [13]. There exist interactions between glucose metabolism and oxygen tension effect to modulate the metabolic shift between glycolysis and OXPHOS. For example, in low oxygen environment, glycolysis was promoted in a glucose concentration-dependent manner [14,15]. The important role of amino acid metabolism, especially threonine, in maintaining PSC pluripotency through regulation of histone methylation was also recently discovered [16]. More importantly, iPSC reprogramming from somatic cells to pluripotent cells has demonstrated a metabolic shift from OXPHOS to glycolysis, providing the important approach of using metabolic regulation to enhance the reprogramming efficiency [13]. Hence, the characterization of energy metabolism before and after stem cell differentiation and the discovery of molecules regulating metabolic transition will aid the development of efficient and safe therapeutics based on stem cells [17].

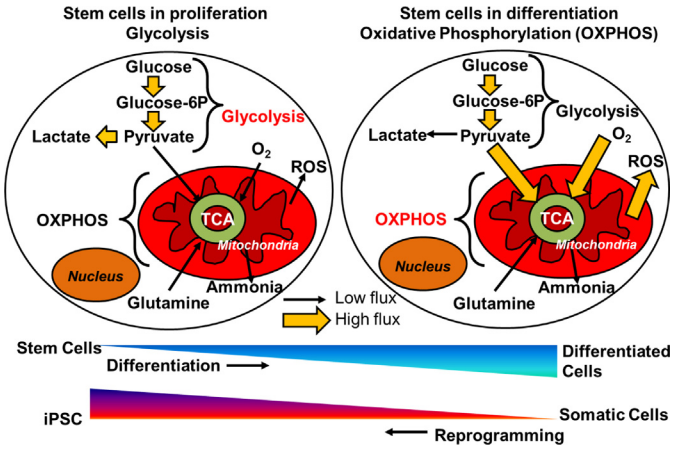

Fig. 1. Schematic illustration of metabolism switch for stem cell proliferation and differentiation. (A) During stem cell expansion, the metabolism relies dominantly on glycolysis, during which glucose is converted to lactate. Low oxygen tension favors glycolysis and the production of reactive oxygen species (ROS) is limited. (B) During stem cell differentiation, the metabolism relies dominantly on OXPHOS: glucose is metabolized through the tricarboxylic acid (TCA) cycle. High oxygen tension is required and may lead to increased ROS production, which acts as a secondary messenger for differentiation. During induced pluripotent stem cell (iPSC) reprogramming, the metabolic switch from OXPHOS to glycolysis is observed.

To fulfill the unique potentials of MSCs and PSCs in clinical applications, large amounts of stem cells in a functional state are required (up to 10^{10}–10^{12} cells per production batch for high dose therapy) [18]. Bioreactors can provide a physiologically controlled environment and the dynamic and temporal delivery of growth factors and nutrients to promote stem cell proliferation and differentiation towards large-scale production [19]. Stem cell metabolites can be used as potent regulators and effective indicators during large scale production in bioreactors. However, the translational potential of incorporating MSC and PSC metabolism at various stages of differentiation in the design of efficient culture processes in bioreactors has not been investigated in detail. In addition, the thorough understanding of metabolic shift upon stem cell differentiation is required to exploit the potential of well-instrumented bioreactors.

This review analyzes the current understanding of MSC and PSC metabolism during expansion and differentiation as well as the emerging role of metabolic plasticity in iPSC reprogramming. In addition, the translational potential of incorporating stem cell metabolism into the design of efficient culture processes in bioreactors is discussed towards mass production of stem cell-derived therapeutics.

2. Glycolysis and oxidative phosphorylation in stem cells

2.1. MSC metabolism

Central metabolism has been shown to play a critical role in MSC expansion. Consistent with the Warburg effect, MSCs in proliferation are dependent on the glycolysis pathway rather than on OXPHOS to meet their requirements in biosynthesis and bioenergy storage [20,21]. During MSC expansion, the OXPHOS pathway has been shown to contribute to only 30% of ATP generation [22]. The culture conditions that affect the shift between glycolysis and OXPHOS are found to impact MSC survival, proliferation, and differentiation. It has been shown that low oxygen tension (i.e., hypoxia) promoted MSC glycolysis [21], decreased reactive oxygen species (ROS) generation [23], and sustained MSC stemness and life span by reducing senescence (Fig. 2) [10,22]. The cells can sense the decrease in oxygen tension to activate the cellular response dependent on mitochondrial-generated ROS but independent of OXPHOS [24]. The addition of the glycolysis inhibitor 2-deoxyglucose led to significant cell death of MSCs, indicating that glycolysis contributed to MSC survival in ischemic condition [21]. Consistent with the Crabtree effect, a reduced glucose concentration in the culture medium reduced lactate production from MSCs without changing total ATP production, compared to the condition with high glucose concentration in the culture medium (Fig. 3) [10]. Therefore, oxygen tension, glucose, and the metabolism-associated biomolecules are important regulators for MSC expansion.

Central metabolism has been also shown to play an important role in MSC differentiation. Adipogenic differentiation of MSCs correlated with the enhanced utilization of OXPHOS as well as with oxygen uptake [25,26]. To induce adipogenic differentiation,