METABOLIC REGULATION OF FUNCTIONAL DECLINE DURING *IN VITRO* EXPANSION OF HUMAN MESENCHYMAL STEM CELLS

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Human mesenchymal stem cells (hMSCs) isolated from various adult tissues are primary candidates in cell therapy and being tested in clinical trials for a wide range of diseases. The pro-regenerative and therapeutic properties of hMSCs are largely attributed to their trophic effects that coordinately modulate the progression of inflammation and enhance the endogenous tissue repair by host progenitor cells. However, immediately after isolation and upon culture expansion, hMSCs lose their *in vivo* quiescent state and start to accumulate genetic and phenotypic changes that significantly alter their phenotypic properties with reduced clonogenic population and therapeutic potential [1]. The culture-induced changes lead to both cellular senescence and metabolic alteration, resulting in reduced therapeutic outcome in various disease models. Since clinical application requires defined cellular properties and large-scale production of hMSCs, preserving cellular homeostasis during hMSCs *in vitro* expansion is a major barrier for hMSCs-based therapy and production. Once viewed as a mere consequence of the state of a cell, metabolism is now known to play active roles in regulating cellular events that govern stem cell phenotype and age-related functional properties during *in vitro* culture. Replicative passaging of hMSCs leads to cellular senescence following with insufficient energy production, decline of stemness and functional properties.

Here, we report that energy metabolism in regulating hMSC aging-related properties due to *in vitro* replicative culture expansion in 2D planner or spinner flask bioreactor. hMSCs under *in vitro* culture up to 15 passages exhibited higher senescence with significant morphological alteration. ^{13}C-glucose-based GC-MS metabolomics analysis suggested that metabolically heterogeneity at low passage hMSCs population while metabolic shift from glycolysis towards OXPHOS at high passage hMSCs. Rapid production of energy required for maintaining cellular properties of hMSCs alters mitochondrial function and leads to breakdown of cellular homeostasis with metabolic and redox imbalance. The alteration of metabolic profile and disruption of cellular homeostasis results in the replicative senescence and decline of therapeutic potentials of hMSCs. Understanding of hMSCs aging during *in vitro* culture expansion provides the insight of metabolic regulation for stem cell fate and engineering aspects for preserving and rejuvenating hMSCs functions via 3D culture or restore of metabolic balance [2].

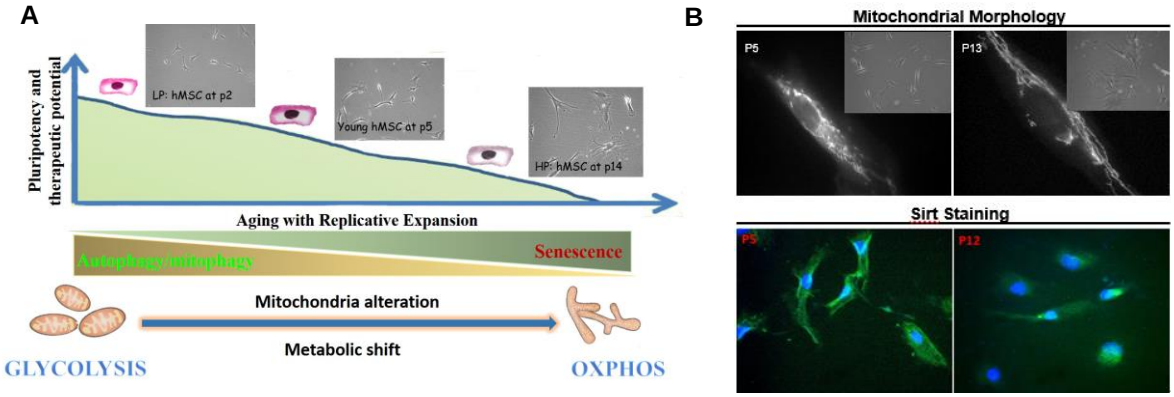

Figure 1 – hMSCs cellular function decline during replicative culture expansion with metabolic alteration (A), mitochondrial alteration and Sirtuin expression (B).

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