論 文 内 容 要 旨

The improved performance of mesenchymal stem cell cultures by fibronectin and mixed self-assembled monolayers under serum-free conditions

(無血清培養下でのフィブロネクチンと混合自己組織化単分子膜による間葉系幹細胞培養系の性能改善)

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(Introduction)

In cell culture and tissue engineering, biomaterials, such as fibronectin (FN), as well as certain synthetic chemical surfaces, promote cell adhesion. FN has been widely used to enhance adhesion and spreading of various cells. Self-assembled monolayers (SAMs) are chemically defined culture surfaces expressing functional groups and have been used as a model surface in studies on adhesion and proliferation of various cells. Previous studies have shown that, among various SAMs, a SAM expressing hydroxyl and carboxylic acid groups at 60:40 (mixed SAM) is optimal for proliferation of mesenchymal stem cells (MSCs) in a serum-free medium. Serum-free media are useful for safe and reliable *ex vivo* expansion of MSCs in regenerative medicine, although MSCs are poorly adhesive in serum-free media on conventional plastic tissue culture plates (TCP). The aims of this study are to improve adhesion and proliferation of MSCs in a serum-free medium by using FN-coated plates and the mixed SAM and to characterize initial cellular responses to these surfaces by DNA microarray and metabolomic analyses, which may give a new insight into culture surface-MSC interactions after cell seeding.

(Methods and materials)

Human bone marrow-derived MSCs were seeded on various surfaces with a serum-free medium STK2. Cell number was estimated using MTT assays or counted with Burker-Turk 30 min-18 days after seeding. Cell spreading was estimated by measuring cell area using Image-J software 2 and 24 h after seeding. For osteogenic differentiation, MSCs were exposed to a serum-free osteogenesis-induction medium STK3 for 4-14 days. The osteogenic potential was estimated by real time PCR analysis of several bone-related genes and alizarin red staining. DNA microarray analysis was performed using Agilent Whole Human Genome Oligo microarrays. Metabolomic profiles in MSCs were determined by liquid chromatography-mass spectrometry (LS-MS).

(Results)

Coating with FN enhanced adhesion of MSCs at 30, 60, and 120 min, markedly increased cell size at 2 and 24 h, and enhanced proliferation for 6-18 days in STK2 compared to TCP. The mixed SAM also enhanced adhesion and proliferation, but did not increase cell size compared to TCP. On FN and the mixed SAM, bone-related genes, such as alkaline phosphatase liver type (*ALPL*), runt-related transcription factor 2 (*RUNX2*), osteocalcin (*OCN*), and osteopontin (*OPN*), were up-regulated 4, 8, and 12 days after exposure to STK3. Alizarin red staining showed that MSC cultures on FN and TCP are calcified at similar high levels after exposure to STK3. These findings revealed that FN and the mixed SAM enhance adhesion and proliferation of MSCs and maintain their osteogenic

potential under serum-free conditions.

Next, we examined whether FN and the mixed SAM may alter global gene expression profile in MSCs. FN did not substantially alter the gene expression profile at 24 h, and no specific gene groups were selectively up-regulated or down-regulated on FN in Gene Ontology analysis. In contrast, the mixed SAM markedly altered global gene expression prolife, and many (158 and 5) gene groups were selectively down-regulated and up-regulated, respectively, in Gene Ontology (GO) analysis. Among them, a group of mitochondria-related genes, including *Cox7b, Cox7c, NDUFB3, NDUFS4* and *UQCRB*, is markedly down-regulated on the mixed SAM. The down-regulation of these genes was confirmed by real time PCR analysis. Furthermore, in metabolomic analysis, the 2-oxoglutaric acid (2-OG) level and the lactate/pyruvate ratio were higher on the mixed SAM than on TCP in all examined MSC lines (n=3), suggesting reduced mitochondria tricarboxylic acid (TCA) cycle activity and enhanced anaerobic glycolysis.

(Discussion and conclusions)

FN and the mixed SAM improved the performance of serum-free MSC cultures. Therefore, both surfaces will be useful in *ex vivo* expansion of MSCs and tissue engineering. However, FN and the mixed SAM had distinct effects on cell size/spreading and global gene expression. FN markedly increased cell size relative to TCP but had little effect on global gene expression profile. Therefore, FN does not compromise MSC-characteristic gene expression profile, and is easily applicable to tissue engineering. On the other hand, the mixed SAM did not increase cell size, but altered global gene expression profile: On the SAM, many mitochondria-related genes were down-regulated, TCA cycle activity was suppressed, and anaerobic glycolysis was enhanced. This finding is interesting, because mitochondria plays a crucial role in the maintenance of pluripotency of embryonic stem cells and induced pluripotent stem cells. Our findings also demonstrate that DNA microarray and metabolomic analyses provide valuable information on interactions between transplantable cells and biomaterials/scaffolds/synthetic surfaces.