論文の内容の要旨

論文題目

Improving multipotent differentiation efficiency of mesenchymal stem cells using the 3D spheroids method on micropatterned substrates

（3Dスフェロイドによる骨髄間葉幹細胞の高効率かつ多機能性な分化方法の確立）

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This dissertation is an attempt to improve stem cell differentiation efficiency via a novel 3D spheroid culture method.

Easily to be isolated and facing no ethical considerations, it is well known that MSCs have tremendous therapeutic potential in regenerative medicine due to their plasticity of differentiation into multiple cell lineages. But the low differentiation efficiency of MSCs to adult cell, which is caused by the traditional 2D culture and differentiation methods use in current MSCs research, limits MSCs to go further in present reconstructive medicine. To overcome the limitations low efficiency, the 2D method should be improved. Although 3D culture methods using highly porous, biomimetic scaffolds could reproducibly increase stem cell differentiation efficiency over their 2D counterparts, these 3D porous scaffolds are also very limited in creating a very precisely controlled microenvironment for stem cell differentiation.

Thus a novel 3D multicellular spheroid culture system based on photolithography micropatterning techniques was introduced in my research. This system produces substrates which are patterned with micrometer-sized domains of predefined geometric features to culture multicellular spheroids of uniform quality and density, and thus might be applied to increase the stem cell differentiation efficiency. On micropatterned substrates coated with special photosensitized poly(ethylene glycol) (P-PEG), 3D multicellular spheroids of MSCs in precise and uniform quality were prepared first. Then MSCs spheroids were differentiated into adipocytes and osteoblasts by adding the corresponding stimuli to check the multipotent differentiation efficiency of spheroid culture MSCs. The morphological results and the measurement of specific RNA expression levels revealed that, in comparison to the conventional 2D monolayer culture method, the 3D spheroid culture method could greatly improve the differentiation efficiency in a precise and quantifiable way.

To identify transcriptional signatures of specific stem cells and to gain insights into mechanisms regulating stem cells differentiation, we applied global gene microarray analysis method. The gene microarray data confirmed that 3D spheroid condition was superior to 2D monolayer condition by more strongly stimulating global gene expression during MSC

differentiation to adipocytes and osteoblasts, which are broadly consistent with those of previously mentioned microscopic observation and PCR tests. In further, Gene microarray date suggested that the 3D culture system offers great promise for directing stem cell differentiation in vitro. MSCs induced in 3D spheroid culture system might enhance global gene expression not only by directly increasing the genes related ECM, cell-cell interaction, growth factors, but also by down-regulating the expression level of stemness maintenance genes.

After confirming the improvement of the differentiation efficiency of the 3D culture method, we carried out further research on the differentiation of MSCs to more specific functional adult cells of hepatocytes, in order to move the 3D culture system closer to clinical applications. Results show the specific genes expression of ALB and CYP1A1 in spheroid differentiation group achieved higher level than monolayer group, demonstrating that 3D spheroid culture not only improve the differentiation efficiency, but also improved the specific functions of liver.

From the results attained in this study, it can be concluded that the 3D spheroid culture system offers a controllable platform for improve the differentiation efficiency of stem cells to adult cells. Furthermore, since this 3D spheroid culture system can make cells form into multicellular spheroid precisely and homogeneously, it can also be used for liver tissue engineering to improve the functional status of differentiated hepatic cells from stem cells.