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Mini Review

Immortalized mesenchymal stem cells producing conditioned medium in a large scale for therapeutic usage

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Stem cells participate in tissue repair not only by generating daughter cells as new cellular components of the tissue being reconstructed but also by secreting biologically active molecules such as cytokines and growth factors to recruit and stimulate other types of cells. Recent studies demonstrated that the conditioned medium of mesenchymal stem cell culture can enhance tissue repair in animal models. In order to produce the conditioned medium of mesenchymal stem cells of constantly high quality in a large scale, we have generated immortalized clones of human mesenchymal stem cells. The immortalized cells displayed similar molecular signatures to those of primary mesenchymal stem cells as indicated by gene expression profiling analysis, and are expected to serve as a stable source of conditioned medium for therapeutic usage. The conditioned medium of the immortalized mesenchymal stem cells is compatible with mass production and mass distribution, and therefore can become available globally at reasonable costs. Since it is also compatible with allogeneic administration, the number of patients benefited by this invention shall be enormous.

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Introduction

Regenerative medicine aims at repairing or replacing damaged tissues[1]. Transplantation of somatic stem cells (e.g., mesenchymal stem cells: MSCs)[2] or of cells derived from pluripotent stem cells [e.g., induced pluripotent stem cells: iPS cells[3] and embryonic stem cells: ES cells[4]] is an attractive approach to achieve the goal. However, several issues need to be addressed for the successful therapeutic use of stem cell transplantation. Firstly, there is a potential risk of malignant transformation of transplanted cells. Secondly, there is a risk associated with surgical operations of transplantation, or a risk of pulmonary embolism when infusing cells intravenously. In applications where stem cells need to be cultured before administration, optimal culture conditions and skilled handling of cells are required to maintain the cells in a constantly good condition. Extra caution is needed when stem cells are transferred between a cell processing facility and a clinic. Lengthy procedures in cell preparation and cell culture may make it difficult to treat patients of an urgent need (e.g., acute or subacute phase of cerebral infarction or spinal cord injury). Additionally, cells need to be prepared individually for each patient leading to costly treatment.

Conditioned medium of stem cell culture as a therapeutic tool to repair damaged tissues

Since stem cells and progenitor cells generate and secrete biologically active molecules that enhance tissue repair in a paracrine fashion[5], some of the issues described above may be circumvented by administering such active molecules contained in the conditioned medium (CM) of stem cell culture, without transplanting cells. This approach of using CM makes the preparation and handling of the therapeutic agents easier, and allows the mass production and mass distribution of the therapeutic agents. Moreover, they can be used in cases of urgent need while keeping per sample costs low. Furthermore, allogeneic treatment with CM will not be hampered by graft rejection unlike the transplantation of allogeneic cells or organs.

Recent studies have been demonstrating the therapeutic potential of CM from MSCs (MSC-CM) in repairing damaged tissues. For instance, CM of human dental pulp-derived stem cells was shown to promote functional recovery in a rat model of spinal cord injury[6]. CM of human exfoliated deciduous tooth-derived stem cells enhanced recovery of rats from focal cerebral ischemia[7]. CM of human bone marrow-derived MSCs promoted bone regeneration in a rat model[8]. Active molecules secreted from MSCs can also be used for treating wounded skin, liver disease, and diabetes mellitus[9-11]. Therefore, administration of MSC-CM can be an effective therapy for repairing damaged tissues while circumventing some of the issues associated with cell transplantation described above.

Immortalized human mesenchymal stem cells as a stable source of therapeutic conditioned medium

The use of MSC-CM, however, has issues of its own. For generating MSC-CM as a therapeutic agent to treat many patients, it is critical to maintain its quality with little lot-to-lot variability. This is challenging when working with primary MSCs, which undergo a limited number of cell division and change the phenotype in long-term culture. In order to resolve the issue, we immortalized human dental pulp-derived MSCs by introducing immortalizing genes [human papillomavirus (HPV) E6 and E7 genes and hTERT gene] to primary MSCs, and investigated whether the dental pulp-derived immortalized MSCs (DP-iMSCs) sustain the molecular characteristics of primary MSCs in their gene expression profiles.

When the gene expression profiles of DP-iMSCs and primary MSCs were compared using gene expression

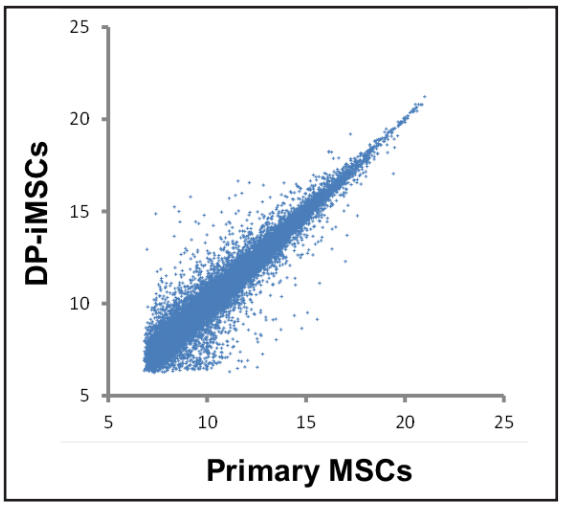

Fig. 1 Signal intensities on gene expression microarrays comparing DP-iMSCs and primary MSCs

Signal intensities were normalized and presented as log-scaled arbitrary values. DP-iMSCs and primary MSCs showed similar gene expression profiles with the correlation coefficient of 0.96.

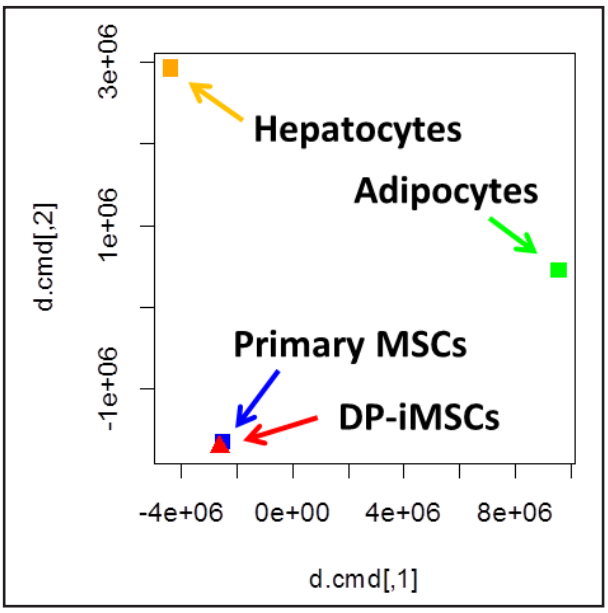

Fig. 2 Multi-dimensional scaling plot comparing the gene expression profiles of DP-iMSCs and other cell types

Using the expression levels of all genes on the gene expression microarrays, the molecular profiles were compared between DP-iMSCs (red triangle), primary MSCs (blue square), adipocytes (green square) and hepatocytes (orange square). The distance matrix was displayed using multi-dimensional scaling. Note the close proximity between DP-iMSCs and primary MSCs.

microarrays (SurePrint G3 Human GE 8x60K v2 Microarrays, Agilent Technologies), the two types of cells demonstrated similar gene expression profiles with the correlation coefficient of 0.96 (Fig. 1). Dozens of genes encoding biologically active molecules (cytokines and growth factors) were commonly expressed in both types of cells. Differentially expressed genes between them were analyzed for their biological functions using DAVID (http://david.abcc.ncifcrf.gov/) to reveal that the most significant difference resides in a set of genes with the Gene Ontology term of "regulation of cell proliferation." This is consistent with the idea that they are primarily different in the property of cell proliferation.

To better recognize the similarity between the cell types, they were further compared with other types of cultured human cells. Four types of human cells (DP-iMSCs, primary MSCs, primary hepatocytes, and adipocytes differentiated from white preadipocytes) were cultured and characterized using gene expression microarrays for their gene expression profiles. As displayed in a multi-dimensional scaling plot (Fig. 2), DP-iMSCs and primary MSCs showed almost identical profiles while they were quite distinct from adipocytes or hepatocytes, indicating the close similarity between DP-iMSCs and primary MSCs.

Conclusion

In conclusion, we immortalized human dental pulp-derived MSCs to generate DP-iMSCs, which maintain the molecular characteristics of primary MSCs. DP-iMSCs are expected to generate a large quantity of MSC-CM while maintaining the characteristics of MSCs over time. DP-iMSCs make the mass production and mass distribution of MSC-CM feasible, and the MSC-CM can be administered in an allogeneic manner to treat many patients around the globe. Since stem cells secrete various biologically active molecules, careful monitoring of adverse effects is essential for the clinical application of MSC-CM as well as of cell transplantation. Depending on the nature and stage of the disease to be treated, suitable strategies can be taken using either tactics: MSC-CM, cell transplantation, or both.

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Conflict of interests

There is no conflict of interest to be disclosed.

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