学 位 論 文 の 要 旨

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学　位　論　文　名	Morphology-based Noninvasive Early Prediction of Serial-passage Potency Enhances the Selection of Clone-derived High-potency Cell Bank From Mesenchymal Stem Cells
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論 文 内 容 の 要 旨

INTRODUCTION

Rapidly expanding clones (RECs) are one of the single-cell-derived mesenchymal stem cell clones sorted from human bone marrow mononuclear cells (BMMCs), which possess advantageous features. The RECs exhibit long-lasting proliferation potency that allows more than 10-repeated serial-passages in vitro, considerably benefiting the manufacturing process of allogenic MSC-based therapeutic products. Although RECs aid the preparation of large-variation clone libraries for a greedy selection of better-quality clones, such a selection is only possible by establishing multiple-candidate cell banks for quality comparisons. Thus, there is a high demand for a novel method that can predict "low-risk and high-potency clones" early and in a feasible manner given the excessive cost and effort required to maintain such an establishment.

MATERIALS AND METHODS

LNGFR and Thy-1 co-positive cells from BMMCs were single cell sorted into 96 well plates, and only fast-growing clones that reached confluency in two weeks were picked up and passaged as RECs. Fifteen RECs were prepared as passage 3 (P3) cryostock as the primary cell bank. From this cryostock, RECs were passaged until their proliferation limitation; their serial-passage limitation numbers were labeled as serial-passage potencies. At the P1 stage, phase-contrast microscopic images were obtained over 6–90 h to identify time-course changes of 24 morphological descriptors describing cell population information. Machine learning models were constructed using the morphological descriptors for predicting serial-passage potencies.

The time window and field-of-view-number effects were evaluated to identify the most efficient image data usage condition for realizing high-performance serial-passage potency models.

RESULTS AND DISCUSSION

Serial-passage test results indicated variations of 7–13-repeated serial-passage potencies within RECs. Such potency values were predicted quantitatively with high performance (RMSE < 1.0) from P1 morphological profiles using a LASSO model. The earliest and minimum effort predictions require 6–30 h with 40 FOVs and 6–90 h with 15 FOVs, respectively.

RECs are clonal MSCs selected from human BMMCs, which not only retain the superior-qualities of conventionally processed MSCs, but also have characteristics that are advantageous for practical cell manufacturing process for therapeutic products. In particular, the high proliferative potency of RECs provides a major advantage in developing efficient manufacturing processes for cell therapeutic products. Therefore, in this study, we investigated the possibility of predicting continuous passaging capacity from initial morphological information alone and its most practical construction method so that the capacity of RECs can be evaluated from the initial stage of cell bank construction.

A long unsolved problem in any type of cell culture is determining "the best timing to make cryo-stocks" in the expansion culture. Since most normal cells lose their proliferation potency when cultured in vitro, one can only bet on which passage number to end with while making cryo-stocks. For establishing industrial cell banks, such a betting factor amounts to a significant risk: the low-success-rate expansion culture will incur significant expenses if the cell does not proliferate as expected, and if the cells are cryo-preserved too early, the stock will not profit production efficacy. In practice, the bottleneck in the practical cell bank establishment is the effort of recruiting precious donors, and not the effort of making greedy selection of candidate cell banks. However, with RECs, we expect a stricter and more selective process for finalizing the candidate cell bank as a "master cell bank." Our investigation presents a new concept of using morphological noninvasive analysis as an "in-process analysis tool" for enhancing and optimizing the cell bank establishment process. This concept will help set the best cryo-stock production timing by balancing "the yield of cells" and "the remaining potency of banked cells" and predicting the future serial-passage potency. Such an approach will help discard the present cell bank design concept, which restricts the passage number using data-less logic.

Our morphology-based future-potency prediction on RECs triggers the discussion of whether this developed model can be applied to other MSC cell-bank establishment studies. Currently, we consider that our model is still limited to predict the evaluate RECs. This interpretation is not attributed to the difference in potencies in RECs and bulk MSCs because we

clearly found that the morphological distribution of RECs differs from that of bulk MSCs. Our image-based detailed morphology measurement indicated that the major population of RECs comprises nearly 2-fold smaller cells compared to the bulk MSCs. Such large size differences would make the prediction model structure, the combination of morphological descriptors, fit for RECs because we use "mean and SD" for reflecting the cell population distribution for our morphological profiles. Furthermore, it is practically difficult for bulk MSCs to conduct "serial-passage tests" for as long as RECs. Such large differences between the native potencies of RECs and bulk MSCs can result in unexpected data bias that can unexpectedly develop serial-passage prediction models that discriminate "RECs or bulk MSCs" from biased data. Therefore, it is a future challenge to investigate such universal morphological characteristics that can be reflected in other stem cells.

CONCLUSION

We successfully developed a noninvasive morphology-based machine learning model to enhance the efficiency of establishing cell banks with single-cell-derived RECs for quantitatively predicting the future serial-passage potencies of clones. Conventional methods that can make noninvasive and quantitative predictions without wasting precious cells in the early stage are lacking; the proposed method will provide a more efficient and robust cell bank establishment process for allogenic therapeutic product manufacturing.

Although our findings are based on a limited number of clones, our investigation of image-based machine learning models was found to introduce a new concept of data-driven process management for a more effective cell bank establishment. Our next challenge will be to expand our morphology-based early cell potency predictions to obtain clones with higher differentiation potencies which closely relate to the therapeutic effects.