Strategy for the Production of High Quality Cells

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High quality cells especially of embryonic stem cells are essential for regenerative cell engineering. The properties of each living cell, however, are different one by one even if the cells are cultured in a same medium. Their differences are thought to be caused by interactions between neighbor cells during cell growth and differentiation. Our strategy is the dynamic analysis of target single-cells by femtoinjection of perturbation factors into living cells in a dish or in living tissues. The factors include marker dyes, vectors, siRNA, proteins, drugs, nanoparticles, and their mixture. Specific gene expression, metabolic activity, viability, and morphological properties are candidates of cell quality indicators.

What we should do is to find or develop non-invasive and real time probes associated with respective quality indicators. Fluorescent glucose is an example of such a probe of metabolic activity. Electrostatic and/or dielectric property is an example that is potentially correlated with viability. In these single-cell analyses, femtoinjection is a key technology. Owing to the development of single-cell manipulation supporting robot (SMSR), injection into ES cells can be performed at 100-200 cells/h. The success rate of physical introduction exceeds 80% and the success rate of gene expression reaches 20%. Semi-quantitative introduction of a vector is possible by changing the vector concentration in a injecting pipette at a range 1-1000 ng/μL. The quantity of the vector actually introduced into a single-cell is estimated at a femto g range.

Information obtained by the SMSR-aided single-cell analysis is of great use from the viewpoint of the production of high quality cells. Therefore much higher throughput of SMSR is intensively required. Some challenges accomplished or under way towards its higher performance will be presented in this lecture.

References

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