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 **CARDIAC FUNCTION AND HEART FAILURE****MOUSE CARDIAC STEM CELL LINES IMMORTALIZED WITH HTERT POSSESS DIFFERENT CELL LINEAGE POTENTIALS**

ACC Poster Contributions

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Background: Resident cardiac stem cells (CSCs) that have potential differentiating into cardiomyogenic and endothelial cell lineage are promising candidates for cell therapy in cardiovascular field. However, a drawback is their limited life span during in vitro culture. Therefore, we constructed the retroviral vector expressing human telomerase reverse transcriptase (hTERT)-IRES eGFP to establish immortalized CSC lines.

Methods: Retroviruses were produced in 293GPG packaging cells by transfection with retroviral vectors carrying the hTERT-IRES eGFP genes. CSCs were isolated by collagenase treatment from heart of ICR mice and infected with retroviruses harboring the hTERT-IRES eGFP genes. The putative CSC lines were selected in the medium containing puromycin for about 3 months, and sorted on a fluorescence-activated cell sorter by strong eGFP expression. The two hTERT-immortalized CSC lines derived from a single cell were finally selected in 96-well cell culture plates, and their cellular characteristics and multi-differentiation potential in vitro were evaluated.

Results: They showed strong eGFP expression and high levels of hTERT activity as well as retained as normal diploid karyotypes after an extended culture period more than 4 months. The two CSC lines expressed CD29, CD44, CD71, CD106 and Sca-1 but not CD14, CD34, CD45, CD90, CD117 and CD133. Interestingly, the one CSC line is negative for CD31 whereas the other one is positive for CD31. They retained multi-potential differentiating into cardiomyocytes, endothelial cells, adipocytes and osteocytes. However, the CD31⁺/Sca-1⁺CSC cell line showed high endothelial differentiation potential compared to the CD31⁻/Sca-1⁺CSC line as determined by Real-time PCR and immunostaining with endothelial cell specific markers as well as capillary network formation by Matrigel assay.

Conclusions: The hTERT-immortalized cardiac stem cell lines with different phenotypic characteristics have different potential for specific cell lineages. The hTERT-immortalized cardiac stem cell lines are very useful for the study of stem cell differentiation as well as for cell and gene therapy in cardiovascular field.