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Tissue preservation, quality controls and aliquoting methods in biobanking

Vincenzo Canzonieri, Silvia Cervo, Tiziana Perin, Paolo De Paoli and **Agostino Steffan**
CRO Aviano National Cancer Institute, Italy

CRO-Biobank (the Biobank of the National Cancer Institute CRO Aviano) is a structured facility integrated in a clinical setting aimed at collecting human biological samples for cancer research. Our mission is to organize a collection of frozen and Formalin Fixed Paraffin Embedded (FFPE) tissue samples with associated biomaterials (e.g. serum, plasma, buffy coat, and nucleic acid). Frozen tissue samples are OCT-embedded, flash frozen in liquid nitrogen and stored at -80°C. For clinical diagnosis, molecular analysis and biobanking of paraffin-embedded tissues, we are developing a specific experience on different histological fixatives, alternative to formalin, which is toxic and carcinogenic. We use well-defined methods to control tissue quality: Histocytology (frozen sections, FFPE mirrored samples and cytological imprint) and molecular pathology. As quality control of DNA, RNA and protein extracts, we randomly perform molecular analyses using appropriate positive and negative controls. Based on the model of "Expert Centers", a specific activity of biological characterization of solid and hematological tumors has been planned in our Institution, using aliquots of samples stored in our biobank. NGS, proteomic and immunohistochemical preliminary analyses will provide selected series of highly informative biosamples to the researchers. In order to optimize the use of biological samples, we have elaborated an "aliquoting sytem" that allows to select the appropriate quantity of material delivered for a single project and may warrant appropriate selection of cells for research purposes. Moreover, we are implementing quality processes and procedures to guarantee the safety and privacy of the providers and to ensure both the traceability and the quality of samples. We also consider ethical, legal, social issues (ELSI) and IT topics, taking into account indications from the Common Service ELSI established in 2015, as a key asset of BBMRI-ERIC.

vcanzonieri@cro.it

Mesenchymal stem cells obtained from synovial fluid mesenchymal stem cell-derived induced pluripotent stem cells on a Matrigel coating exhibited enhanced proliferation and differentiation potential

Yangpeng Sun
Sun Yat-sen University, China

Induced pluripotent stem cell-derived mesenchymal stem cells (iPSC-MSCs) serve as promising source for cell-based therapies in regenerative medicine. However, optimal methods for transforming iPSCs into MSCs and the characteristics of iPSC-MSCs obtained from different methods remain poorly understood. In this study, we developed a one-step method for obtaining iPSC-MSCs (CD146+STRO-1+ MSCs) from human synovial fluid MSC-derived induced iPSCs (SFMSC-iPSCs). CD146-STRO-1-SFMSCs were reprogrammed into iPSCs by transduction with lentivirus-mediated Sox2, Oct-3/4, klf4, and c-Myc. SFMSC-iPSCs were maintained with mTeSR1 medium in Matrigel-coated culture plates. Single dissociated cells were obtained by digesting the SFMSC-iPSCs with trypsin. The dissociated cells were then plated into Matrigel-coated culture plate with alpha minimum essential medium supplemented with 10% fetal bovine serum, 1× Glutamax, and the ROCK inhibitor Y-27632. Cells were then passaged in standard cell culture plates with alpha minimum essential medium supplemented with 10% fetal bovine serum and 1× Glutamax. After passaging *in vitro*, the cells showed a homogenous spindle-shape similar to their ancestor cells (SFMSCs), but with more robust proliferative activity. Flow cytometric analysis revealed typical MSC surface markers, including expression of CD73, CD90, CD105, and CD44 and lack of CD45, CD34, CD11b, CD19, and HLA-DR. However, these cells were positive for CD146 and stro-1, which the ancestor cells were not. Moreover, the cells could also be induced to differentiate in osteogenic, chondrogenic, and adipogenic lineages *in vitro*. The differentiation potential was improved compared with the ancestor cells *in vitro*. The cells were not found to exhibit oncogenicity *in vivo*. Therefore, the method presented herein facilitated the generation of STRO-1+CD146+ MSCs from SFMSC-iPSCs exhibiting enhanced proliferation and differentiation potential.

sunyp@mail2.sysu.edu.cn