Abstract of Doctoral Dissertation

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Study of Morphological Difference in Hydrogel Induced Cancer Stem Cell in Synovial Sarcoma Model Cells
（滑膜肉腫モデル細胞におけるハイドロゲル誘導幹細胞の形態学的解析）

Cancer is one of the major causes of death all over the world. About 90% cancer related death are due to the metastasis cancer. Radiation, Chemotherapy and/or surgery are the most common treatment of cancer. Cancer tissues are composed of the heterogenous population with small number of CSCs or Cancer stem-like cells, progenitor cells, and majority of differentiated non-CSCs. Where most cancer treatment are designed to target the rapidly dividing cancer cells, the slow-cycling quiescent CSC remain intact and cause therapy-resistance and recurrence in cancer patients. Nevertheless, the identification of CSCs remains as a challenge owing to only few markers to detect CSCs and their limited abundance less than 1%. Thus, development of more effective drugs targeting CSCs and the analyses of the characteristics should be urgent issue for complete eradication of cancer cells from human body, contributing to improvement of prognoses of cancer patients. Therefore, efficient, and highly reliable detection system of CSCs is necessary. In this study, we examine the effect of PAMPS gel as a cultured substrate, mainly focusing on changes of cell morphology and the elevation of the stemness associated gene to identify the morphological characteristics of stem cells in synovial sarcoma model cells.

Polymer hydrogel are being widely used in cell biology. Conventional cell culture materials include polystyrene and glass which may not be suitable to mimic the extracellular matrices that is native in many soft tissues, whereas polymer hydrogels could be adopted for their various mechanical, structural, and compositional alteration in the cell function. Previous study with biomaterial revealed that DN hydrogels composed of PAMPS and PDMAm could efficiently reprogram differentiated cancer cells to CSCs, designated as the HARP phenomena, which is an efficient way to understand CSCs in vivo. We also demonstrated morphological changes of cancer cells with elevation of stemness markers when the cells were cultured on the DN gel. Thus, the hydrogels might provide three-dimensional microenvironment which mimics extracellular matrix to provide specific environment for CSCs.

Cell morphology contributes to diagnosis of various diseases, grade of cancer, disease progression, and treatment responses. Variation in morphology linked to underlying molecular events in disease mechanism which determine the cellular response to different external stimuli such as alteration of microenvironment. Morphological changes are driven by the cytoskeletal reformation in response to various external environmental changes, local information as well as different cell types. It has been shown that using actin cytoskeletal information along with morphological information, it is possible to detect the difference between cancer and non-cancer cells. In addition, morphological information in cancer cells can also give information about characteristics in vivo and associated genes along with the cell shape determinants can also differ significantly between the different morphological colonies. Morphological changes have also been reported for the stem cell undergoing the differentiation process in several studies. Furthermore, morphological characteristics of stem cell also provides the characteristics of the lineage determination in their differentiation process.

Synovial sarcoma is an aggressive mesenchymal neoplasm that mainly presents in the extremities of adolescents and young adults, and accounts for 5% to 10% of all soft tissue sarcoma. The cause of this sarcoma is formation of chromosomal translocation t(X:18; p11:q11), resulting in the fusion gene SSX-SS18. Oncoprotein SSX-SS18 is involved in chromatin remodeling leading to epigenetic changes by replacing naïve SS18, an organizing component of the SWI/SNF or BRG1/BRM-associated factor (BAF) complex. It also causes transcriptional changes by cross-linking polycomb repressive complex 2 (PRC2) to activating transcription factor 2 (ATF2). The necessity to understand how the translocation and elevated biomarkers interact with the host is still unknown. Further research is required for development of greater understanding of these interactions and the downstream effects that occurs in synovial sarcoma. This will enable us to monitor patients for progression of disease and to develop better treatments to completely cure this subtype of soft tissue sarcoma.

In this study, we examined on the essential elements for morphological changes that occurs during reprogramming from non-CSCs to CSCs regarding the HARP phenomena, using features extracted from single cell level. First, murine myoblast C2C12 and its SS18-SSX1-transduced synovial sarcoma model cells (C2C12-SS18-SSX1) were cultured on the PAMPS gel, a component of the DN gel, and confirmed the PAMPS gel-induced stemness along with morphological alterations. The rate of induction of stemness was higher in the sarcoma model cells than wildtype cells. Fluorescence images for CSC like cells such as Sox2 and actin and segmented single cells from fluorescence images were utilized to understand the relationship between cell morphology and the stemness induction. At first, using geometrical feature computed from the single cell we used an unsupervised dimensional reduction to examine if the features can represent associated shapes of the cells. Together with their Sox2 intensity, we identified that cells expressing highly Sox2 exhibit more small rounder shape, whereas the flat polygonal cells possess significantly low Sox2 expression on hydrogel. Taken together, the results demonstrate that there is a clear correlation between the morphological changes on hydrogel and the stemness elevation.