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論　文　内　容　の　要　旨

氏　名　（　李　梦露（MENGLU LI）　）	
論文題名	Study on the progression of human embryonal rhabdomyosarcoma in the cell sheet system（細胞シート系におけるヒト胎児型横紋筋肉腫の挙動に関する研究）

論文内容の要旨

This thesis focused on the study of rhabdomyosarcoma progression in a 3D multicellular system constructed by cell sheet technology. It consists of five chapters including general introduction (chapter 1) and concluding remarks (chapter 5).

Firstly, tumor cell migration in myoblast sheet was investigated. In chapter 2, human embryonal RMS cells (RDs) were co-cultured with myoblast sheet as target cells. Vertical migration was measured as distribution of target cells in the vertical direction of cell sheet. More RDs were found to locate in the upper layer of sheet compared with myoblasts. Horizontal migration rate was calculated by time-lapse observation. The value of RD migration was 7.35 ± 0.12 μm/h, while the migration rate of myoblasts was 5.07 ± 0.06 μm/h. RDs demonstrated a fast migratory phenotype compared with surrounding myoblasts both in vertical and horizontal direction.

Secondly, disruptive effect of RMS cells on cell sheet structure was investigated. After acquiring migration ability, tumor cells will invade their surrounding environment to seek a way out. In chapter 3, deformation of sheet structure was only observed when RDs were co-cultured with myoblasts at a low ratio. Further exploration revealed that tumor cells can adapt to their surrounding environment, keep a highly motile feature and remodel the environment, while myoblasts lost migration ability when facing a comparably dynamic environment. RDs disordered the alignment of myoblasts in the sheet culture and finally disrupted the whole structure of cell sheet.

Finally, the effect of disordered tumor-containing cell sheet on the behaviors of endothelial cells was studied. Tumor-containing cell sheets with a disrupted ECM structure and elevated sheet fluidity promoted migration of endothelial cells and increased the frequency of endothelial disconnection. Early degradation of the endothelial network was observed in the tumor-containing cell sheet. This chapter investigated how the environment change caused by minor RDs affected endothelial network formation.

In conclusion, the three important steps of RMS progression were studied in this research. The direct role of tumor cells in tissue remodeling and subsequent influence on angiogenesis was studied, bringing new insights into the field. This study firstly applied cell sheet technology in the *in vitro* study of cancer, revealing the advantages of this technology in the field. In future, drug screening system will be developed based on cell sheet technology and more cancer types will be tested using this method.

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論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

This thesis focuses on the study of rhabdomyosarcoma progression in a 3D multicellular system constructed by cell sheet technology. It consists of five chapters including general introduction (chapter 1) and concluding remarks (chapter 5).

To initiate metastasis, tumor cells will acquire high motility and detach from the original tumor site. In chapter 2, migration ability of human embryonal RMS cells is studied in the 3D human skeletal muscle myoblast (HSMM) sheet. The analysis of cell migration is divided into vertical migration and horizontal migration to understand migration in a 3D format. Vertical migration of RDs is quantified according to the distribution of RDs in each layer from the bottom to top. It is found that more RDs locate towards the upper layer of cell sheet, demonstrating that RDs migrate faster in vertical direction compared with myoblasts. Horizontal migration of target cells is tracked by time-lapse observation and migration rate of RDs is calculated to be 7.35 μm/h, which is 1.5 times faster than that of myoblasts (5.07 μm/h). RDs demonstrate a fast migratory phenotype compared with surrounding myoblasts both in vertical and horizontal direction.

After acquiring high motility, tumor cells will invade into their surrounding environment to seek a way out. The heterotypic interaction between tumor cells and host tissue cells plays a vital role in this process. In chapter 3, the heterotypic cell-cell interaction is investigated by co-culturing malignant RMS cells with normal myoblasts in a cell sheet. Heterogeneous cell sheet is prepared by mixing different ratios of RDs with HSMMs. The deformation of sheet structure is only observed when RDs are co-cultured with myoblasts at a low ratio; whereas,

homogeneous HSMM or RD sheets maintain the intact structure. Further exploration reveals that tumor cells can adapt to their surrounding environment, keep a highly motile feature and remodel the environment, while myoblasts lose migration ability when facing a comparably dynamic environment. A small population of RDs (10%) are capable to disorder the alignment of myoblasts in the cell sheet and finally disrupt the structure of whole cell sheet. It indicates that minor tumor cells can affect the bulk tissue and suggests that muscle disruption could be initiated by RMS invasion.

Tumor angiogenesis, which is new blood vessel formation in the tumor region, is an important trigger of metastasis by providing oxygen and nutrients to the starving primary tumor. In chapter 4, a tumor-containing cell sheet is prepared by mixing RDs with HSMMs at a ratio of 8% to avoid deformation of sheet structure and co-cultured with green fluorescence protein expressing human umbilical vein endothelial cells (GFP-HUVECs) to understand the effect of tumor cells on endothelial behaviors. The structure of tumor-containing cell sheet differs from the HSMM sheet without tumor cells in disordered fibronectin alignment and elevated sheet fluidity. Interestingly, in highly fluidic tumor-containing cell sheet, the vertical migration of GFP-HUVECs is promoted, finally resulting in the degradation of endothelial network formation. Unlike the conventional study of tumor angiogenesis, which focusing on the cytokines secreted by tumor cells, this chapter studies how the environment change caused by minor RDs affects endothelial network formation. It indicates that disorganized tissue structure facilitates tumor angiogenesis by activation of endothelial cell migration.

This study investigates the progression of RMS in a 3D multicellular system constructed by cell technology. The effect of minor tumor cells on tissue remodeling and subsequent influence on angiogenesis process is studied, bringing new insights into the field.