Breast tumor cell behaviors on in vitro models mimicking extracellular matrix at different malignant stages

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Statement of Purpose: Extracellular matrix (ECM) is composed of many proteins and saccharide to influences many cellular processes such as cell attachment, proliferation, survival, differentiation, and cell migration. The composition of ECM is different among types of tissues, developmental stages, and pathological stages. In tumor progression, ECM composition dynamically changes according to their malignancy. To investigate the roles of ECM in tumor progression, knocked-down animals and cells and protein-coated substrates were used. However, it is difficult to understand comprehensive ECM roles during tumor progression in these researches. *In vitro* models mimicking *in vivo* ECM at different malignant stages will be useful for comprehensive study of ECM in tumor progression. In this study, we prepared in vitro models mimicking ECM in tumor tissue at different malignant stages as "staged tumorigenesis-mimicking matrices" by *in vitro* tumor cell culture and decellularization technique. Moreover, breast tumor cell functions on staged tumorigenesis-mimicking matrices were investigated.

Methods: Breast tumor cell lines, MDA-MB-231 (invasive cancer), MCF-7 (non-invasive cancer), and MCF-10A (benign tumor) were cultured on tissue culture plate (TCPS) for 1 week in DMEM/F-12 (1:1) medium contained with 10% FBS. The expression patterns of ECM genes were compared by RT-PCR analysis. For the decellularization, the cells were treated with Triton X-100 and NH_4OH for 5 min followed by DNase I and RNase A treatment. Cell removal was confirmed by the observation of cell nuclei and intracellular actin filaments using fluorescent microscope. Remained ECM proteins were detected with coomassie brilliant blue (CBB) staining after decellularization. Attached cells on the matrices were counted in three randomly selected fields to compared cell attachment activity of the matrices. Cell proliferation activity on the matrices and the effect of anti-cancer drug on cell proliferation were examined by WST-8 assay.

Results: We compared the ECM gene expression patterns among MDA-MB-231, MCF-7, and MCF-10A cells. *LAMA3* was strongly expressed in MDA-MB-231 cells, which is consistent with previous report. The *LAMA5* expression levels in MDA-MB-231 and MCF-7 cells were higher than in MCF-10A cells. *FN1*, *TNC*, and *POSTN* were strongly expressed in MCF-10A cells. *FN1* and *TNC* were moderately expressed in MDA-MB-231 cells. These results are coincident with previous report. These results indicate that the ECM expression patterns are different among tumor cells at different malignant stages. For the preparation of these ECM proteins deposited beneath the cells as staged tumorigenesis-mimicking matrices, cellular components were removed from the culture by decellularization treatment. After decellularization, nuclei and actin filaments were hardly observed, indicating that cells were successfully removed. Moreover, proteins were detected even after decellularization, suggesting that ECM proteins were remained on TCPS. These results suggested that staged tumorigenesis-mimicking matrices were successfully prepared.

As the first step for the comparison of cellular functions on staged tumorigenesis-mimicking matrices, cell attachment activities of the matrices were examined. All cells examined can adhere on the matrices. Compared among staged tumorigenesis-mimicking matrices, cells showed weak attachment on the matrices derived from MDA-MB-231 cancer cells.

As a next step, cell proliferation activity was compared among staged tumorigenesis-mimicking matrices. The proliferations of MDA-MB-231 and MCF-7 were promoted on the matrices derived from MDA-MB-231 cancer cells whereas MCF-10A cell proliferation was not promoted. MCF-10A cell proliferation was promoted on the matrices derived from MCF-10A cells.

Finally, we compared chemoresistance of MDA-MB-231 cells on staged tumorigenesis-mimicking matrices. Chemoresistance of MDA-MB-231 cells against 5-fluorouracil (5-FU) increased on only matrices derived from MDA-MB-231 cells (Fig. 1).

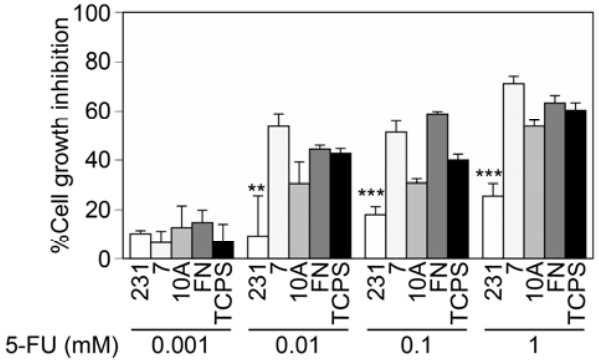

Fig. 1: Chemoresistance of MDA-MB-231 cells on staged tumorigenesis-mimicking matrices. 231, 7, and 10A indicate the matrices derived from MDA-MB-231, MCF-7, and MCF-10A, respectively. FN indicates fibronectin. Data represent means±SD. (n=3)

Conclusion: Our results showed that the cells showed different behaviors on staged tumorigenesis-mimicking matrices according to the malignancy of cell sources for ECM preparation. Therefore, staged tumorigenesis-mimicking matrices might be a useful in vitro ECM models to investigate the roles of ECM in tumor progression.

References: Hoshiba T. Biochem Biophys Res Commun. 2013; 439: 291-296.