DVC Measurement on the Deformation Field of ECM Surrounding a Tumor Spheroid During the Invasive Progression

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Introduction. Cancer infiltration plays an important role in cancerous metastasis. The infiltration indicates that tumor cells proliferate gradually and make a focus at a primary lesion, then the focus extends to the surrounding organs and tissues. Extracellular matrix (ECM) which mainly consists of collagen fibers making complicated fibrous structures, is usually surrounding the tumor focus. Tumor cells in the focus initiate the infiltration due to the conformation change of the ECM. It has, however, been fully verified yet about the mechanical interaction between the tumor focus and the ECM. Therefore, measuring the deformation field of the ECM surrounding the tumor focus must give important tips to understand the cancer infiltration and metastasis. In the present study, a tumor spheroid was employed instead of a tumor focus, the 3D deformation field of ECM was measured using a digital volume correlation (DVC) method with time, and then the mechanical interaction was discussed.

Experimental procedure. Tumor spheroids of almost 150 μm in diameter were made using tumor cells of human pancreatic adenocarcinoma, PANC-1. The PANC-1 cells were three-dimensionally cultured in the gel composed of type I collagen. When the gels with PANC-1 cells were fabricated, two types of specimens were prepared. One is mixed with fluorescent beads, the other is mixed with immunofluorescent collagen fibers. The gels with the fluorescent beads were used for observation of the deformation field using DVC analysis. The gels with the immunofluorescent collagen fibers were used for observation of the collagen conformation. A drop of the 150-μl solution was put onto a glass-based dish, then it turned into a gel in a 37 °C incubator for 1 h.

After the gelation of the specimen, the tumor spheroids, the fluorescent beads and the immunofluorescent collagen fibers were photographed using a confocal laser scanning microscope every 15 min for 2 h. Finally, they were also captured again after adding surface-activating agent, Triton X-100, to the gel. Triton X-100 killed the PANC-1 cells in the gel, then we could obtain the three-dimensional images of the gel with force-free state. The deformation field of the ECM surrounding the PANC-1 cells was analyzed using DVC method as the gel with the force-free state is a standard point. We also evaluated the density of the collagen fibers in the vicinity of the tumor spheroids from the volumetric images of the immunofluorescent collagen fibers of the gel.

Results and Discussion. Figure 1 shows the representative deformation field of ECM surrounding a tumor spheroid 2 h after the observation initiation. The arrows in the figure represent the displacement direction of the ECM, and the colors indicate the magnitude of the displacement. The black volume in the center of the figure represents a tumor spheroid of PANC-1. As the results, it has been found that the deformation of the ECM was dominantly headed to the center of the tumor spheroid. However, the magnitude of the displacement was not uniform, the deformation field of the ECM is quite heterogeneous. This result could be ascribable to the local inhomogeneity of the density of the PANC-1 cells and the collagen fibers.

We calculated the divergence field of the ECM from the displacement field. The divergence was almost zero in areas away from the tumor spheroid, but the ECM around each front edge of the infiltration in the tumor spheroid has a high negative divergence field. It means that the tumor cells at the front edge of the infiltration strongly pull the collagen fibers of the ECM for invasion. According to the high negative divergence field, we confirmed the density of the collagen fibrils at each front of the infiltration from the configuration of the collagen fibrils images. We obtained that it becomes higher as the strength of the divergence field of ECM.

Conclusion. We succeeded to obtain the three-dimensional deformation field of ECM surrounding a tumor spheroid of PANC-1. The deformation field was quite heterogeneous and the negative divergence fields were located at the respective front edges of the infiltration. According to the negative divergence, the collagen fibrils of the ECM were collected at the front edges. We obtained one of the interesting tips with respect to the relationship between the deformation field of ECM and the conformation of ECM surrounding a tumor spheroid.

Fig. 1 3D displacement field of ECM surrounding a tumor spheroid of PANC-1.