In Vitro Tumor Invasion and Metastasis Assays Using 3D-Human Tumor Models with Blood- and Lymph-Capillary Networks

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Statement of Purpose: Development of tumor-targeting drugs for chemotherapy is a central challenge in the pharmaceutical field. However, it is difficult to obtain tissue responses from 2D-monolayered cell models, and also in vivo animal models have limitations for low reproducibility and species difference. In particular, animal testing for cosmetics and chemicals have been prohibited in EU. Therefore, in vitro 3D-tumor models that reconstitute the living tissues are desired for tumor invasion assays. However, there are no reports on 3D-in vitro tumor invasion models containing blood- and lymph-capillary networks.

We reported a bottom-up approach, termed "cell accumulation technique" [1] which improved our previous method (hierarchical cell manipulation [2]), to develop multilayered thick tissues (>100 μm) by cell coating with nanometer-sized ECM films, fibronectin and gelatin (FN-G) films [3]. The vascularized tissues were successfully fabricated by a sandwich culture of endothelial cells between fibroblast multilayers, as well as lymph-capillary models. In this study, we developed novel tumor invasion models with blood- and lymph-capillary networks constructed by the cell-accumulation technique (Figure 1a). We found invasion behavior depended on cancer cell types, and also different influences between blood- and lymph-capillaries were confirmed during the invasion. The comparisons to in vivo behaviors were performed using mouse models.

Methods: The 5 x 10^6 cells/mL normal human dermal fibroblasts (NHDF) were alternatively incubated with 0.04 mg/mL FN and G in 50 mM Tris-HCl (pH = 7.4) for 1 min at 30 rpm. After repeating the nine steps of immersion, the $(FN/G)_4FN$ films with about 7 nm thickness were prepared on the cell surface. The FN-G coated NHDF were seeded into a cell culture insert and cultured for 1 day to construct multilayered tissues. In the same manner, human umbilical vein endothelial cells (HUVECs) or human lymphatic endothelial cells (LECs) were sandwiched between 10-layered NHDF tissues to form vascularized tissues. After that, three types of 1 x 10^5 cells /well of human cancer cells (RFP-expressing MiaPaCa-2, BxPC3, and HT29) were seeded onto blood-capillary models to make invasion models.

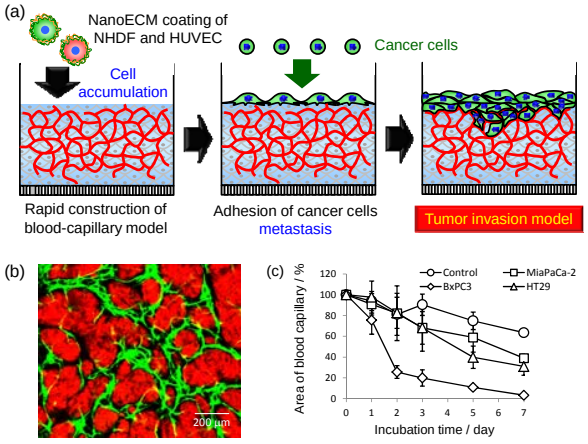

Figure 1. (a) Schematic illustration of the fabrication of 3D- tumor invasion models with blood- and lymph-capillary networks. (b) CLSM image of vascularized HT29 tumor models stained with an anti-CD31 antibody. (c) The area of blood capillary of each tumor model.

Results: We observed high invasion property in MiaPaCa-2 and BxPC3 by histological evaluations (Figure 1b). With regard to the effect on HUVEC tubular networks, HT29 displayed highly vascularized structures (Figure 1c), while blood capillaries in BxPC3 model disappeared during the invasion. Interestingly, BxPC3 models maintained LEC tubular structures and only BxPC3 cells invaded into the LEC tubes (Figure 2a,b). These results suggested that this tumor invasion model can recreate tumor invasion and metastasis process through lymph vessels like in vivo. Comparing the results of invasion and the effects to blood vessels between in vitro and in vivo models, we confirmed the similarity to living tissues and usefulness of these models.

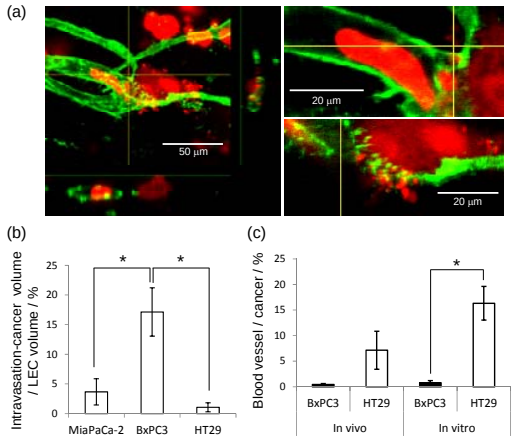

Figure 2. (a) CLSM images of the engineered BxPC3 invasion model with lymph capillaries (CD31: green). (b) Percentage of the volume of intravasation-cancer in each model. (c) Percentage of the area of blood vessel for cancer cells in in vivo and in vitro models. *p<0.05, n=3.

Conclusions: We demonstrated reconstruction of in vitro 3D-tumor invasion and metastasis models and found the similar phenomena to in vivo animal models. This 3D-tumor invasion model with vasculatures would be useful for drug assessments.

Reference:

[1] Nishiguchi A. et al., Adv. Mater. 2011;23:3506-3510.
[2] Matsusaki M. et al., Angew. Chem. Int. Ed. 2007;46:4689-4692.
[3] Matsusaki M. et al., Adv. Mater. 2012;24:454-474.