In Vitro Tumor Intravasation Assays Using 3D-Human Blood- and Lymph-Capillary Models

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Statement of Purpose: Development of tumor-targeting drugs for the use of chemotherapy is a central challenge in the pharmaceutical field. The beneficial effect and toxicity of anti-cancer drugs need to be carefully assessed in a preclinical trial. 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 from 2013 and the system alternative to animal testing is required. Therefore, in vitro 3D-tumor models that reconstitute the living tissues are desired for tumor invasion assays in the preclinical investigation. However, 3D-in vitro tumor intravasation models containing blood- and lymph-capillary networks that can model the living process has not been reported yet.

We reported a bottom-up approach, termed "cell accumulation technique" [1] to develop multilayered thick tissues (>170 μm) by cell coating with nanometer-sized ECM films, fibronectin and gelatin (FN-G) films. The vascularized tissues were successfully fabricated by a sandwich culture of endothelial cells between fibroblast multilayers, as well as lymph-capillary models [2]. In this study, we developed 3D-tumor invasion models with blood- and lymph-capillary networks constructed by the cell-accumulation technique (Figure 1). We found invasion and intravasation 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.

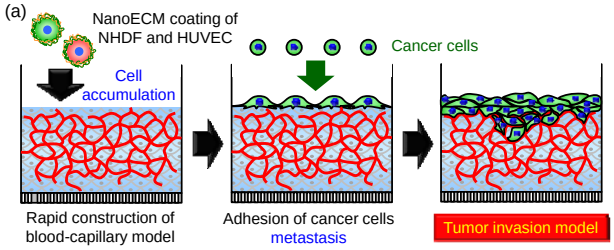

Figure 1. (a) Schematic illustration of 3D- tumor invasion models with blood- and lymph-capillary networks.

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.

Results: We observed high invasion property in MiaPaCa-2 and BxPC3. With regard to the effect on HUVEC tubular networks, MiaPaCa-2 show high intravasation property to HUVEC networks (Figure 2a). On the other hand, blood capillaries in BxPC3 model disappeared during the invasion, while HT29 displayed tumor angiogenesis–like structures (Figure 2b,c). Interestingly, only BxPC3 cells invaded into the LEC tubes (Figure 2d). Since it is known that MiaPaCa-2 spread from blood vessel to induce liver metastasis and BxPC3 tend to cause lymph node metastasis through lymph vessels in living tissues, these results suggested that this tumor invasion model can recreate tumor invasion, intravasation, and metastasis process 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 2e).

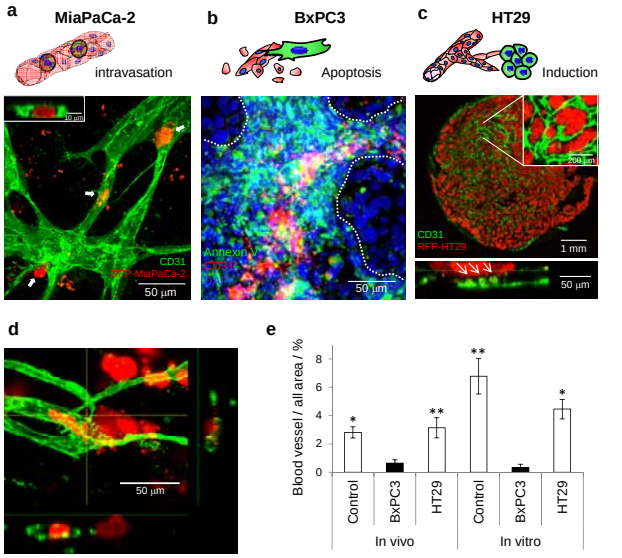

Figure 2. (a-c) CLSM images of the engineered RFP-MiaPaca-2, BxPC3, and RFP-HT29 intravasation models with blood capillaries (CD31 or Annexin V). (d) CLSM images of the engineered RFP-BxPC3 intravasation models with lymph capillaries. (e) Percentage of the area of blood vessel for cancer cells in in vivo and in vitro models. *P<0.05, **P<0.01 when compare with BxPC3 (n=3).

Conclusions: We demonstrated the reconstruction of in vitro 3D-tumor invasion and intravasation 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] Nishiguchi A. et al., Biomaterials 2014;35:4739-4748.