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Technical Note

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Microfluidic co-culture platforms for modeling tumor angiogenesis in alveolar soft part sarcoma

Alveolar soft part sarcoma (ASPS) is a rare and slow-growing soft tissue sarcoma known for its resistance to conventional chemotherapy. A key pathogenic feature of ASPS is its strong angiogenic activity. The tumor forms highly integrated vasculature enriched with pericytes (PCs), which supports both vascular stability and metastatic potential.

In this study, we developed a microfluidic co-culture vasculature chip incorporating ASPS cells, pericytes, and endothelial cells to replicate the angiogenic tumor microenvironment of ASPS. This platform successfully modeled key features of ASPS angiogenesis and reconstructed leaky tumor vasculature, enabling the evaluation of drug penetration into solid tumor cores.

Moreover, this model allowed us to investigate protein-mediated signaling from ASPS cells to PCs and endothelial cells, including the involvement of Rab27a and Sytl2. These findings provide valuable insights for developing targeted therapies that disrupt tumor–stroma communication in ASPS.

Experimental Method

A microfluidic device was fabricated using polydimethylsiloxane (PDMS) and designed with three channels separated by micropillars. To model ASPS angiogenesis, an ASPS spheroid was embedded in a fibrin–collagen I gel and loaded into the center channel. The left and right channels were filled with EGM-2 medium.

For vascular network formation, endothelial cells (ECs) and pericytes (PCs) at a 20:1 ratio were co-injected into the side channels, where they adhered to the surface of the fibrin–collagen I gel. The device was maintained at 37 °C with 5% CO_2, and the medium was refreshed daily. Angiogenesis was initiated on day 0.

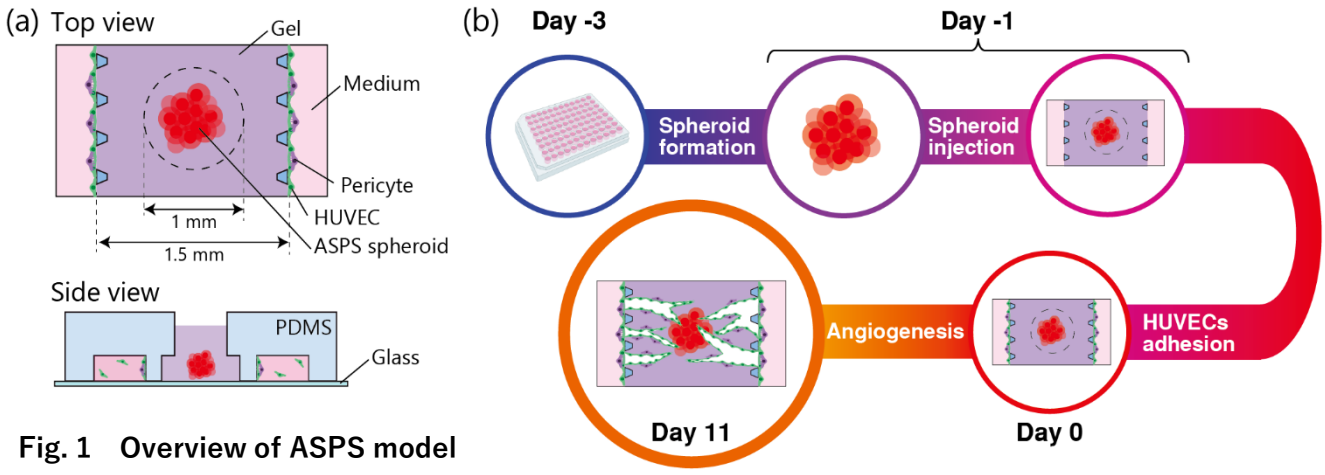

Fig. 1 Overview of ASPS model

Angiogenesis Comparison: Wild-Type vs. Causative Gene–Deficient ASPS Cells

To investigate ASPS angiogenesis in an on-chip environment, 3D spheroids of ASPS cells were used to model solid tumors. Two cell types were compared: ASPS cells expressing the primary causative fusion gene (“ASPS”) and long-passaged ASPS cells that had lost expression of the gene (“Null”). Angiogenic sprouting was monitored over 11 days by tracking green fluorescent signals.By day 7, ASPS spheroids exhibited approximately 80% sprout coverage and extensive invasion, while Null spheroids showed only ~40% coverage and ~10% invasion. These results underscore the strong angiogenic potential of ASPS cells, driven by the presence of the fusion gene.

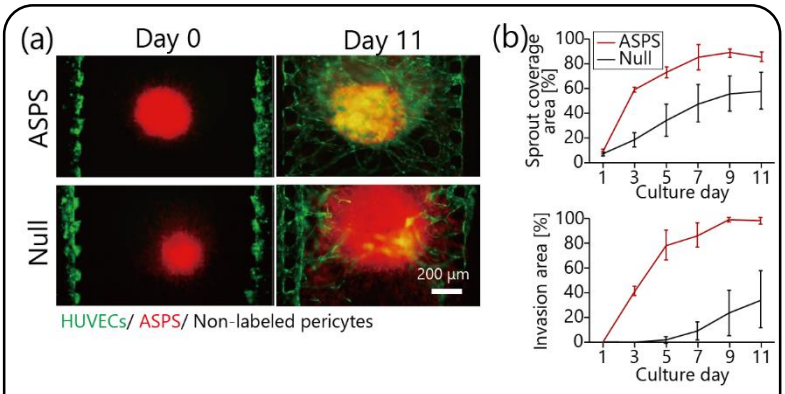

Figure 2. Differences in angiogenesis depending on the presence or absence of the causative gene

a. Representative images of angiogenesis.
b. Quantitative analysis of angiogenesis under each condition (ASPS and Null cells). $n = 5$ devices, Error bars: S.D.

Leaky vasculature induced by ASPS spheroid

Tumor vessel perfusability and permeability were evaluated using ASPS spheroids and compared to non-tumor fibroblast spheroids as controls. BSA-Alexa Fluor 647 dye perfused through tumor-associated vessels within 10 seconds and fully diffused into the tumor spheroid by 600 seconds, indicating high vascular permeability. In contrast, vessels in the non-tumor control retained the dye for over 600 seconds, suggesting significantly lower permeability.

These findings demonstrate that our MPS model successfully recapitulates the leaky vasculature characteristic of tumors, providing a valuable platform for evaluating drug diffusion through abnormal tumor vessels.

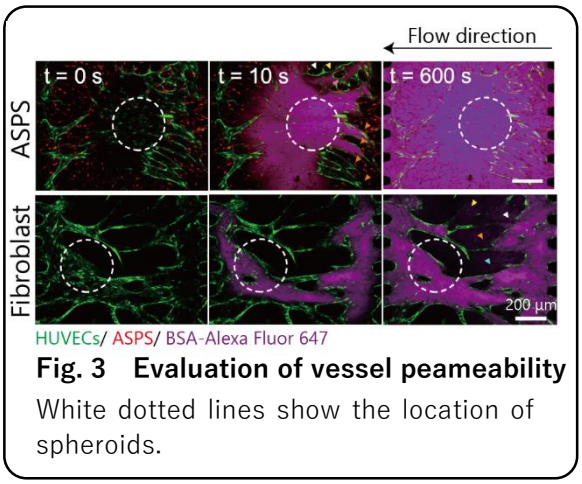

Fig. 3 Evaluation of vessel peameability

White dotted lines show the location of spheroids.

Role of intracellular trafficking

Rab27a and Sytl2 mediate ASPS-induced angiogenesis by trafficking signaling proteins (Pdgfb, Gpnmb, Angptl2) to pericytes and endothelial cells. Knockout of Rab27a or Sytl2 (sgRab27a/sgSytl2) significantly reduced sprout coverage compared to controls, highlighting their critical role in tumor vessel formation. Targeting Rab27a and Sytl2 may inhibit ASPS angiogenesis and aid the development of novel therapies for ASPS.

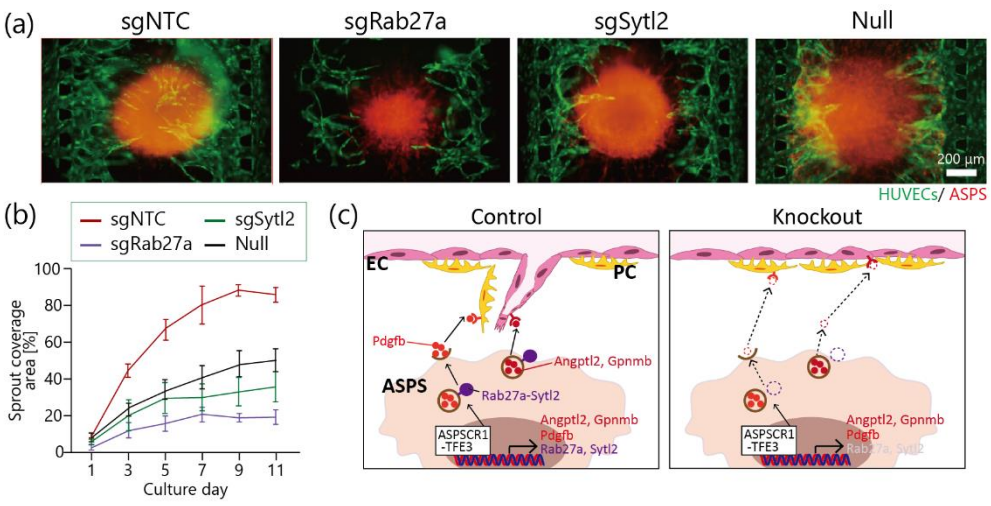

Fig. 4 Evaluation of angiogenesis using knockouot cells

a. Representative images of angiogenesis. sgNTC indicates ASPS cells transduced with non-targeting sgRNA.
b. Quantitative analysis. Sample size: $n = 5$ devices, Error bars: S.D.
c. Proposed mechanism of ASPS-induced angiogenesis.