A MICROFLUIDIC INVASION ASSAY FOR GLIOMA-INITIATING
CELLS IN THREE-DIMENSIONAL CULTURE
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ABSTRACT
This paper presents a microfluidic three-dimensional (3D) platform to investigate behaviors of glioma-initiating cells (GIC) cocultured with human umbilical vein endothelial cells (HUVEC). In this platform, these cells were cultured in microenvironments similar to in vivo. Especially, our experiments have been performed with GIC established by gene transfection [1], which is different from conventional tumor cell lines. Our results demonstrate that biochemical factors secreted by HUVEC attracts glioma invasion.

KEYWORD: Microfluidic device, Glioma-initiating cells, Invasion

INTRODUCTION
Since gliomas invade normal tissues rapidly, most of them are diagnosed at an advanced stage. It is therefore important for therapeutic strategies to understand the characteristics of glioma. However, the process of gliomagenesis is not well understood yet. In vitro assays for glioma have been performed with conventional cell lines in previous studies. However, they didn’t show the similarity to human glioma in vivo. In vivo assays also have some problems on difficult imaging and controlling specific microenvironmental factors. In our previous study, we created a coculture model of tumor cell lines and HUVEC [2]. Here, we used GIC, which are more feasible tumor cells established by gene transfection, and present behaviors of glioma cocultured with HUVEC in the microfluidic 3D platform.

EXPERIMENTAL
Microfluidic platform
Microfluidic devices made of poly(dimethylsiloxane) (PDMS) were fabricated using soft lithography. The device used in this study is made of PDMS cured on a silicon template (Fig. 1). After the PDMS device was exposure to plasma of air, the device was bonded with cover glass to form microfluidic channels between the PDMS and cover glass.

The device has two microfluidic channels for the culture of GIC and HUVEC (Fig. 2). A mixed gel solution of Matrigel and type I collagen was injected into a center channel to form a 3D gel scaffold for cells (Fig. 2A). GIC was injected into one channel at 3.0×10^4 cells/device, while HUVEC was injected into the other channel at 2.0×10^4 cells/device (Fig. 2B). Cells attached to the surface of the mixed gel within 1 day. The invasion process of GIC was observed by a phase-contrast microscope equipped with a time-lapse system until day 7.

GIC-HUVEC coculture
GIC were established by overexpressing H-Ras in normal neural stem/progenitor cells isolated from the subventricular zone of adult mice harboring a homozygous deletion of the Ink4a/Arf locus [1]. Invasion process was compared in serum-containing differentiation medium and in serum-free stem cell medium which keeps property of stem cells to investigate the role of tumor stem cells. HUVEC was seeded at the other channel one day after GIC was seeded.
Fig. 1: The method for making an SU-8 mold and a microfluidic device.

Fig. 2: (A) A schematic image of the microfluidic device with an enlarged image of a gel region. (B) Process of HUVEC-GIC coculture.

RESULTS AND DISCUSSION

3D invasion assay for GIC with HUVEC

The glioma invasiveness was higher in coculture with HUVEC than in monoculture regardless of culture medium, the differentiation and stem cell media (Fig. 3), indicating that the secreted factors from HUVEC promoted the invasion of GIC. Tumor tissues were reported to promote angiogenesis and recruit blood vessels to inside of the tissues. However, our result suggested that GIC itself invaded toward blood vessels.

Single-cell migration and collective migration

When GIC were cultured in the stem cell medium, the cells showed single-cell migration (Figs. 3 and 4). In contrast, when the cells were cultured in the differentiation medium, they showed collective migration (Figs. 3 and 4). Their motility was higher in single-cell migration than in collective migration.
CONCLUSION
We have established a GIC-HUVEC coculture model in a microfluidic device and analyzed the interaction between GIC and HUVEC. We found that GIC showed collective and single-cell invasions depending on their differentiation. In addition, the GIC invasions were enhanced by the presence of HUVEC. This model can be used to further investigate behaviors of cancer stem cells in the process of glioma invasions into 3D matrix.

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