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Recapitulation of cancer stem cell niches in glioblastoma on 3D microfluidic cell culture devices under gravity-driven perfusion

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ABSTRACT

The low survival rate of patients with glioblastoma is in part due to the heterogeneity in the cell population of glioblastoma that includes cancer stem cells (CSCs). CSC niches include a hypoxic core that is also closely linked to self-renewal ability, migration, and drug resistance. Here, we report a CSC culture method in three-dimensional microfluidic cell culture devices under gravity-driven perfusion, which we dub hypoxia chips (H-chips). In H-chips, glioblastoma cells, U87, spontaneously formed spheroids within 12 h, even without any addition of growth factors. Compared to monolayer-cultured cells in dishes, spheroids in H-chips showed higher expression of CSC markers, such as hypoxia-inducible factor-1α (HIF-1α), CD133, and nestin. Spheroids in H-chips were more resistant to doxorubicin than monolayer-cultured ones in dishes. Transcriptional profiling revealed that the expression of interleukin-6 (IL-6), one of the inflammatory cytokines, was higher in spheroids in H-chips than in monolayer-cultured cells in dishes. IL-6 depleted cells failed to form spheroids in H-chips, and their drug resistance decreased. These results suggest that in H-chips, glioblastoma cells increased the production of IL-6, and promoted spheroid formation and other cancer stem cell properties, such as drug resistance. Our microfluidic cell culture method is highly useful for recapitulating CSC niches.

Introduction

Glioblastoma is the most frequent and aggressive central nervous system tumor in humans, and is classified by the World Health Organization (WHO) as grade IV astrocytoma. Patients with glioblastoma have a 5-year survival rate of 2–5% [1]. This low survival rate may be due to the heterogeneity in the cell population of glioblastoma that includes cancer stem cells (CSCs) [2]. CSCs are cancer cells within a tumor that have stem-cell like properties that give rise to all cell types found within the tumors [3]. They are mainly found in the hypoxic niche of glioblastoma in vivo, and are related to chemoresistance and the recurrence of glioblastoma [4]. The hypoxic niche locates near the areas of necrosis, and functions to maintain the CSC population. A hypoxic condition in stem cell niches is necessary to maintain CSCs in an undifferentiated state, and also to minimize DNA damage via oxidation [5,6]. Under hypoxic conditions (between 1.5% to 5%), hypoxia inducible factor-1α (HIF-1α) in CSCs is not degraded, and can trigger a signaling cascade that drives the gene expression of stem cells, and makes CSCs adapt to hypoxic conditions [5]. There are other markers for CSCs, such as CD133 [6], and neuroectodermal stem cell marker (nestin) [7]. CD133 is a cell membrane protein [6], whose expression is regulated by HIF-1α, HIF-2β, etc [8]. CD133 is regulated by p53, and controls the growth and tumor-initiating capacity of CSCs [9]. Nestin is also induced by hypoxia [7]. In addition to these stem cell markers, cytokines, such as interleukin-6 (IL-6) and interleukin-8 (IL-8), and stromal-derived growth factor 1α (SDF-1α/CXCL12) contribute to the construction of CSC niches [10]. In particular, both IL-6 aberrant production and following autocrine IL-6 signaling are closely related to tumor generation and poor disease outcomes in glioblastoma [11,12].

A variety of spheroidal culture tools have been developed, since spheroids are believed to better reflect molecular backgrounds and

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drug responses in vivo, compared to monolayer cells [13–15]. Spheroids can recapitulate the microenvironmental characteristics of solid tumors in vivo, including anchorage-independent growth and concentration gradients that are essential to tumor progression [14]. Anchorage-independent growth requires cell-to-cell junctions that lead to signaling cascades for malignancy. The hypoxic conditions in the spheroidal core often reduce the sensitivity of cells to both radiation and chemotherapy while increasing the population of cancer stem cells that are known to chemotherapy [5]. Conventional spheroid culture tools include low attachment multi-well plates [16], hydrogels [17], and hanging drop [18]. While synthetic hydrogels have the advantage of controlling the gel stiffness, most of their substrates are not suitable for in vivo transplantation [19]. Although low attachment multi-well plates can produce many spheroids, these spheroids form spontaneously, and their size varies. This variation often leads to high variation in drug response from spheroids in low attachment multi-well plates [16]. While the hanging drop generates one spheroid per well, and can control spheroid size, medium replacement is not easy.

Recently, three-dimensional microfluidic cell culture devices (3DμFCCDs) have been used to recapitulate the microenvironment of the tissue and organs in vivo to overcome the limit of conventional spheroid culture tools [20–22]. 3DμFCCDs can be advantageous to culture spheroids, because they can control a small volume of fluid inside the channel, and continue to supply nutrients to cells. In addition, interactions among cells can be studied by separating and inserting hydrogels containing several different types of cells in microfluidic devices [17]. Recently, we have shown that the stemness of human adipose-derived stem cells (hADSCs) was maintained in 3DμFCCDs, when they were cultured at high cell density by gravity-driven perfusion [22]. HIF-1α expression was higher in hADSCs cultured in 3DμFCCDs than in those cultured in Petri dishes under a normoxic condition, suggesting that cell culture in 3DμFCCDs under gravity-driven perfusion is well suited for recapitulating a hypoxic condition in stem cells. Thus, we called 3DμFCCDs 'hypoxia chips' ('H-chips'), in particular when cells were cultured under a gravity-driven perfusion condition.

In this study, we report a microfluidic cell culture method to recapitulate CSC niches of glioblastoma (U87) in H-chips (Fig. 1A). It only needs bovine serum albumin (BSA) coating to prevent cell adhesion to the surface of the glass slide on the bottom of the chip before use. In H-chips, U87 cells formed spheroids within 12 h and could maintain the 3D structure for at least three days. To demonstrate the physiological relevance of the method to recapitulate CSC niches, we compared gene expression pattern, cytokine production, and doxorubicin (Adriamycin, Dox) resistance of U87 cells cultured in H-chips to those of cells cultured in Petri dishes under normoxic condition. When cells grew under gravity-driven perfusion, they increased both expression of CSC markers, such as HIF-1α, CD133, and nestin, and production of IL-6. These cells displayed higher resistance to Dox than those in a Petri dish.

Experimental

Fabrication of H-chips

We modified the fabrication of H-chips (Fig. 1) from our previous 3DμFCCDs [21,22]. In detail, an H-chip consist of two preformed polydimethyl siloxane (PDMS) (Sylgard® 184, Dow-Corning) layers, and a glass slide. In details, the top PDMS layer with two medium replacement chambers (8 mm diameter) and one cell seeding hole (800 μm diameter) (Fig. 1B) was prepared by first curing a mixture of PDMS and its curing agent in 10: 1 ratio (w/w) in a Petri dish and later punching respective holes into the cured 3.5 mm-thick PDMS layer. Photolithography with a negative tone photoresist (SU-8 2100, MicroChem Co., USA) was used to fabricate a master mold with patterns of an array of 30 μm × 50 μm elliptical micropillars with a 20 μm gap size situated in the center of a microchannel (2 cm × 1 mm) (Fig. 1A and C). The middle PDMS layer in 5 mm thick were then peeled off from the mold [21,22]. Once both PDMS layers were ready, the top layer was treated with O_2 plasma for 40 s at 70 W, and then bound to the middle layer, with the channel pattern facing the glass slide (Fig. 1B). In a completed H-chip, the cell seeding hole in the top layer was connected to the center hole in the middle layer, while the medium

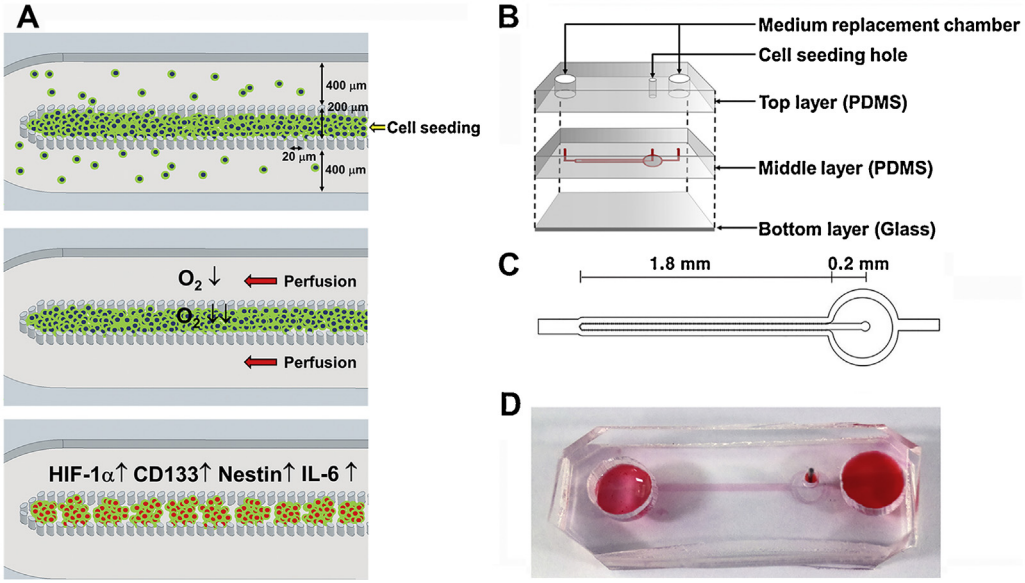

Fig. 1. Design and operation of an H-chip. (A) Schematics of spheroid formation on the H-chip explained by hypoxia and expression changes of the genes encoding HIF-1α, CD133, Nestin and IL-6. (B) Bonding of three layers of the H-chip. (C) Design of the microchannels. (D) A real image of the H-chip filled with red ink.

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