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Human three-dimensional engineered neural tissue reveals cellular and molecular events following cytomegalovirus infection

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ABSTRACT

Human cytomegalovirus (HCMV) is the most common cause of congenital infection of the central nervous system (CNS). To overcome the limited access to human neural tissue and stringent species specificity of HCMV, we used engineered neural tissues to: (i) provide a technical advance to mimick features of HCMV infection in a human neural fetal tissue *in vitro* and (ii) characterize the molecular and cellular phenomenon following HCMV infection in this tissue. Herein, we infected hESC-derived engineered neural tissues (ENTs) whose organization resembles fetal brain. Transcriptome analysis of ENTs demonstrated that HCMV infection displayed features of the infection with the expression of genes involved in lipid metabolism, growth and development, as well as stress and host-response in a time-dependent manner. Immunohistochemical analysis demonstrated that HCMV did not firstly infect neural tubes (*i.e.* radially organized, proliferating stem cell niches), but rather an adjacent side population of post-mitotic cells expressing nestin, doublecortin, Sox1, musashi and vimentin markers. Importantly, we observe the same tropism in naturally HCMV-infected fetal brain specimens. To the best of our knowledge this system represents the first human brain-like tissue able to provide a more physiologically model for studying HCMV infection.

1. Introduction

Human cytomegalovirus (HCMV) is a ubiquitous herpes virus representing one of the most feared intrauterine infections. This fetal infection can lead to central nervous system (CNS) anomalies, with an average incidence of 0.2–2.5% of all live births [1,2]. Clinical symptoms such as intrauterine growth restriction or microcephaly are observed in 10–20% of infected neonates leading to neurodevelopmental damage that leads to nervous system disorders such as mental retardation, motor impairment, sensorineural hearing loss or visual impairment [2–4]. Epidemiological studies estimate that seroconversion to HCMV occurs in 1–4% of pregnant women. Gestational age at infection does not seem to affect the rate of transmission. However, infection in the first half of pregnancy bears a greater risk of symptomatic fetal complications [2,4].

The species specificity of HCMV precludes the direct study of the virus in animal models and prevents studies of the biological events that occur *in vivo* during human fetal brain infection [4–9].

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However, to investigate CMV neuropathogenesis, studies with murine models have been performed [10]. In rodent brains, the early targets of mouse CMV (MCMV) are neural stem/progenitor cells localized in the ventricular (VZ) and subventricular zones (SVZ). This observation has also been confirmed in neurospheres cultured from fetal forebrain [10]. In contrast to neural stem cells (NSC) and neural precursor cells (NPC), mouse embryonic stem cells (ESC) are refractory to MCMV infection and acquire susceptibility during differentiation [11,12]. Other investigators have developed *in vitro* models of HCMV infection of human neural cells and NPCs isolated from aborted fetuses or derived from human embryonic stem cells (hESC) or induced pluripotent stem cells (iPS).

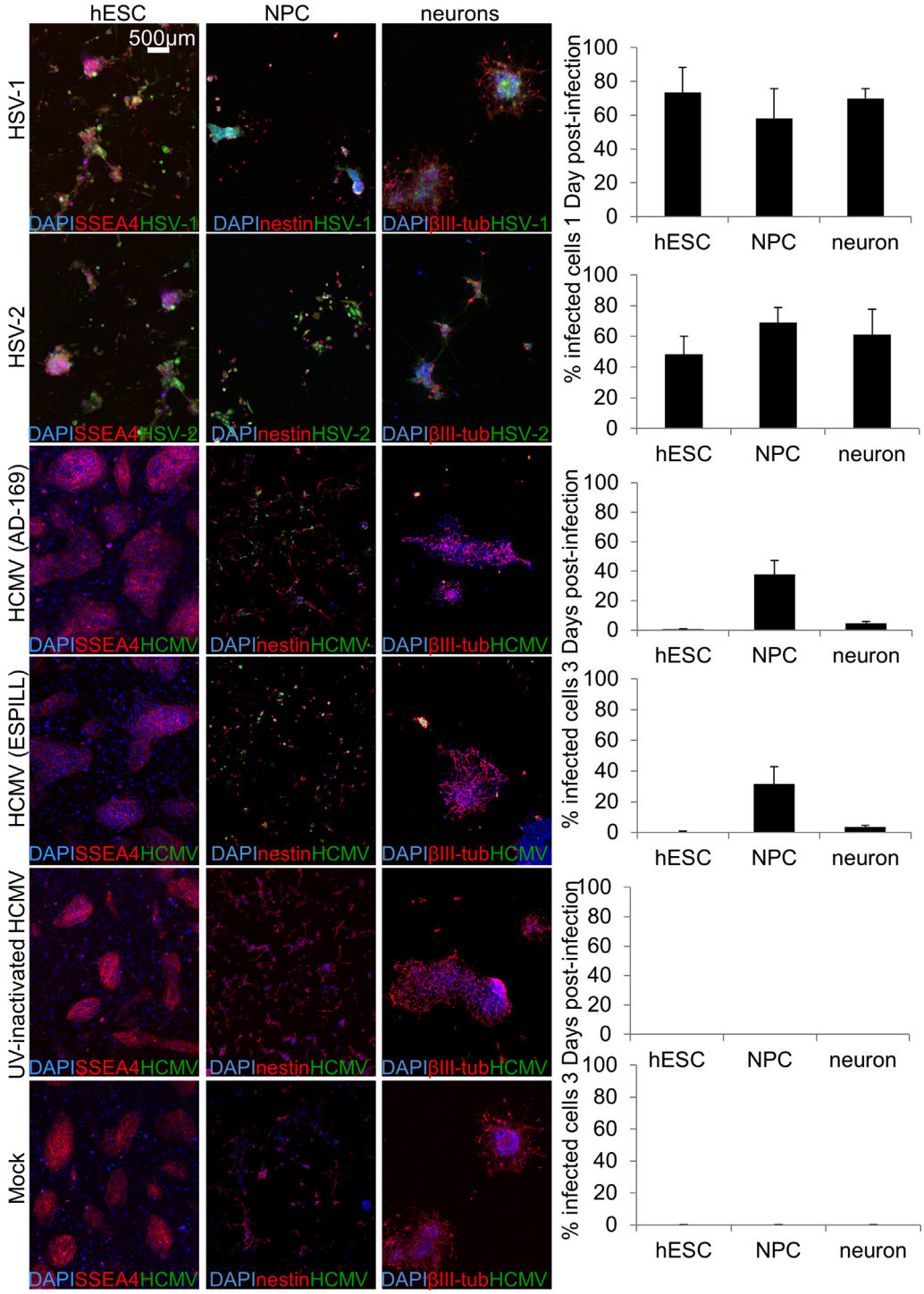

Fig. 1. Infection of human embryonic stem cells (hESCs), neural progenitors (NPCs) and neurons by different herpes viruses. Undifferentiated hESCs, as well as hESC-derived NPCs and neurons were infected with human herpes virus 1 (HSV-1), human herpes virus 2 (HSV-1), as well two strains of human cytomegalovirus (HCMV, strains AD-169 and Espill). Cells were infected for 24 h (HSV-1, HSV-2) or 72 h (HCMV). Mock-infected cells, as well as cells exposed to UV-inactivated HCMV were used as controls. Cells were immunostained with antibodies against SSEA4, nestin, βIII-tubulin, as well as antibodies against the respective viral antigens (HSV-1 and HSV-2 glycoprotein C and HCMV immediate early antigen (IEA). For nuclear counterstaining, DAPI was used. Histograms represent the percentage of cells (hESC, NPC, neuron) that stained positive with antibodies for the respective viruses. Data are represented as mean (n = 3) ±SEM. βIIItub: βIII-tubulin.