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Influence of oxygen tension on CD133 phenotype in human glioma cell cultures

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Abstract

Under standard culture conditions, tumor cells are exposed to 20% O_2, whereas the mean tumor oxygen levels within the tumor are much lower. We demonstrate, using low-passaged human tumor cell cultures established from glioma, that a reduction in the oxygen level in these cell cultures dramatically increases the percentage of CD133 expressing cells.

Keywords: Cell culture; CD133; Glioma; Cancer stem cell

1. Introduction

In numerous tumors or cancer cell lines, cancer stem cells can be isolated either on the expression of CD133 [1–4] or on the basis of their ability to exclude the fluorescent vital dye Hoechst 33342 [5]. However, recent data have raised some concerns about the use of Hoechst 33342 to identify cancer stem cells from cancer cell lines [6–8]. It has been reported that tumor stem cells derived from glioblastomas cultured in serum-free medium more closely mirror the phenotype and genotype of primary tumors than do serum-cultured cell lines [9]. Indeed the use of serum-containing medium in cell culture has long been controversial and is considered as ''non-physiological'' since cells are not usually exposed to serum. In addition to serum, it is claimed that oxygen tension in cell culture is another parameter that does not mirror physiological conditions [10–12]. Standard tissue culture incubator conditions are 5% CO_2 and 95% air [10–12]. Hence, cells are exposed to oxygen tensions close to 20%, whereas mean tissue oxygen is described to be much lower, ranging from 1% to 5% in the brain [10,13]. Thus, according to these data, current standard culture conditions can be said ''normoxic'' with regard to our cell culture conventions (cell culture normoxia or ''cultural'' normoxia) but are indeed hyperoxic with regard to physiological conditions. Although it is clear that cell culture conditions cannot be identical to physiological conditions, deleterious effects of culturing cells at the O_2 concentration

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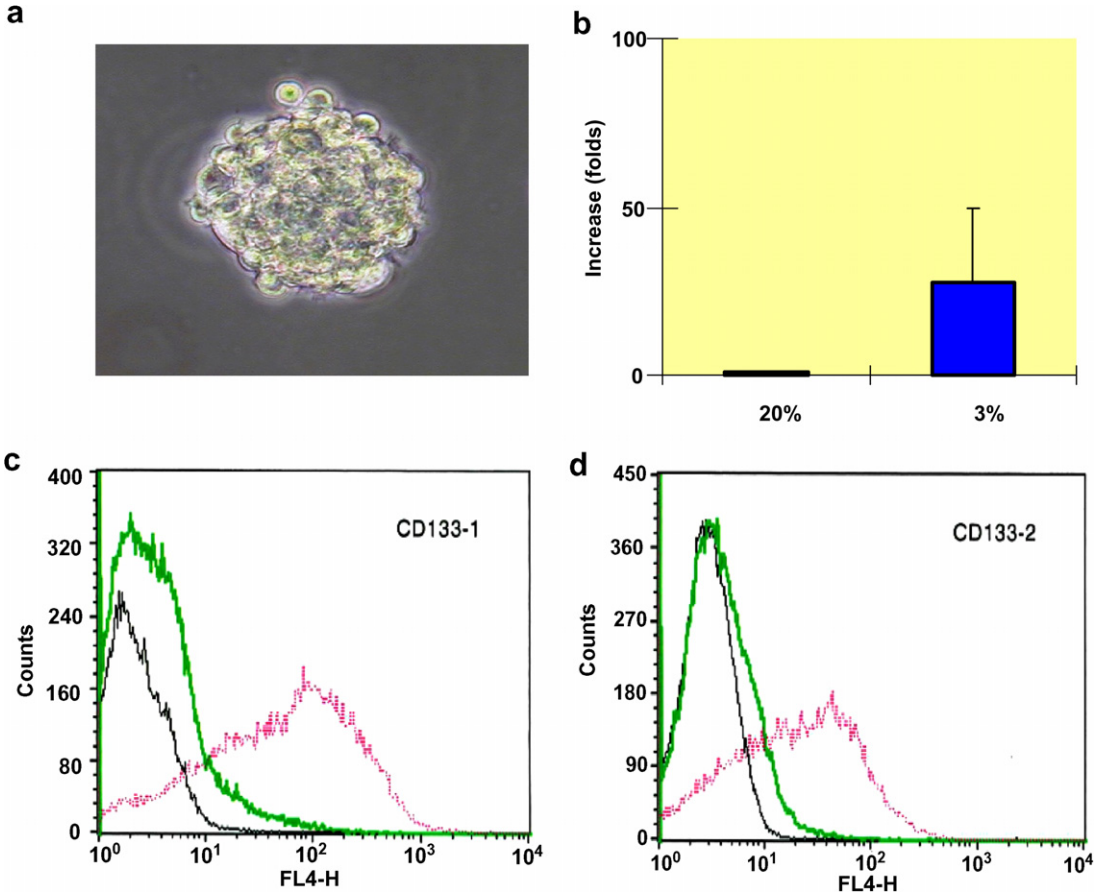

Fig. 1. Cells from human glioblastoma, grow as neurosphere, and have increased CD133 expression under 3% O_2. (a) Glioblastoma cells growing as nonadherent neurospheres. (b) CD133 real time quantitative RT-PCR. Real-time PCRs were carried out with SYBR Green PCR master mix(Qiagen) as described in Section 2. For each sample, gene expression was normalized by the relative expression of the housekeeping gene β-actin (ACTB) which showed a stable expression in any conditions. (c) Flow cytometric analysis for CD133 expression of glioma cells cultured under 20% O_2 (green) or 3% O_2 (red). CD133 monoclonal antibody was CD133/1 (AC133); non-specific negative control antibody is in black. (d) Flow cytometric analysis for CD133 expression of glioma cells cultured under 20% O_2 (green) or 3% O_2 (red). CD133 monoclonal antibody was CD133/2 (293C3); non-specific negative control antibody is in black. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

of air have been reported. For example, human diploid fibroblast cells grown under 3% O_2 achieve more population doublings during their life times than when they are cultured at the O_2 concentration of air (20%) [14]. Likewise, 20% O_2 cell culture conditions alter stem cell function and proliferation [11,15]. Consequently, it can be assumed that cell culture in a "hypoxic" incubator in 3% O_2 more closely mirrors tissular normoxia. Accumulative evidence points to CD133 as a cell marker suitable for identifying cancer stem cells from brain tumors [1,16]. To investigate whether oxygen influences CD133 expression in glioma cell cultures we compared CD133 expression from tumor dissociated cells cultured as tumor-derived sphere in serum-free medium at 20% O_2 or 3% O_2.

2. Materials and methods

2.1. Cell cultures

Tumor samples from three glioblastoma and two anaplastic oligodendroglioma were obtained within 2 h of surgical resection from adult gliomas. Tumor tissue was washed twice in HBSS (Gibco), minced, and enzymatically dissociated for 1 h at 37 °C in a solution of HBSS containing 0.1% dispase (Gibco, 1 u/mg) and 0.01% DNAse (Sigma) with intermittent careful mechanical trituration. Dissociation was sometimes improved by additional 10–15 min treatment with trypsin/EDTA (Gibco, 0.05%/0.53 mM). Alternatively tissue dissociation was performed using NeuroCult® enzymatic dissociation kit from StemCell Technologies. Cells were then collected by centrifugation, resuspended in defined medium consist-