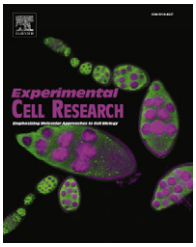
EXPERIMENTAL CELL RESEARCH 318 (2012) 1104–1111

Available online at www.sciencedirect.com

SciVerse ScienceDirect

www.elsevier.com/locate/yexcr



Research Article

Cellular characteristics of head and neck cancer stem cells in type IV collagen-coated adherent cultures

Young Chang Lim[a,1], *Se-Yeong Oh*[b,1], *Hyunggee Kim*[b,*]

[a]*Department of Otorhinolaryngology-Head and Neck Surgery, Research Institute of Medical Science, Konkuk University School of Medicine, Republic of Korea*

[b]*School of Life Sciences and Biotechnology, Korea University, Republic of Korea*

ARTICLE INFORMATION

Article Chronology:
Received 5 October 2011
Revised version received
27 February 2012
Accepted 29 February 2012
Available online 8 March 2012

Keywords:
Cancer stem cell
Head neck cancer
Type IV collagen
Adherent cultures

ABSTRACT

Although head and neck squamous carcinoma cancer stem cells (HNSC-CSCs) can be enriched in serum-free suspension cultures, it is difficult to stably expand HNSC-CSC lines in suspension due to spontaneous apoptosis and differentiation. Here, we investigated whether HNSC-CSCs can be expanded without loss of stem cell properties by adherent culture methods. Cell culture plates were coated with type IV collagen, laminin, or fibronectin. We examined cancer stem cell traits of adherent HNSC-CSCs grown on these plates using immunocytochemistry for stem cell marker expression and analyses of chemo-resistance and xenograft tumorigenicity. We also assessed the growth rate, apoptosis rate, and gene transduction efficiency of adherent and suspended HNSC-CSCs. HNSC-CSCs grew much faster on type IV collagen-coated plates than in suspension. Adherent HNSC-CSCs expressed putative stem cell markers (OCT4 and CD44) and were chemo-resistant to various cytotoxic drugs (cisplatin, fluorouracil, paclitaxel, and docetaxel). Adherent HNSC-CSCs at the limiting dilution (1000 cells) produced tumors in nude mice. Adherent HNSC-CSCs also showed less spontaneous apoptotic cell death and were more competent to lentiviral transduction than suspended HNSC-CSCs. In conclusion, compared to suspension cultures, adherence on type IV collagen-coated culture plates provides better experimental conditions for HNSC-CSC expansion, which should facilitate various refined cellular studies.

© 2012 Elsevier Inc. All rights reserved.

Introduction

Recently, it has been hypothesized that a small subpopulation of tumor cells with "stem cell-like" characteristics called cancer stem cells (CSCs), which are distinct from the bulk of the cancer cells in the tumor, are a principal culprit of tumor initiation, invasion, metastasis, and treatment resistance [1,2]. Initially identified in hematopoietic cancers, a number of CSCs have been isolated from various solid human malignancies, such as brain, lung, breast, colon, ovary, and prostate cancer, and their molecular and cellular features have been elucidated [3–9]. Although a recent study reported isolation of head and neck squamous carcinoma (HNSC)-derived CSCs by fluorescence-activated cell sorting (FACS) using a specific surface marker, CD44 [10], our laboratory has also isolated and characterized HNSC-CSCs using a more effective experimental technique: a stem cell culture-based isolating

* *Corresponding author at:* School of Life Sciences and Biotechnology, Korea University, Seoul 136–713, Republic of Korea. Fax: +82 2 953 0737.
E-mail address: hg-kim@korea.ac.kr (H. Kim).

Abbreviations: HNSC, head and neck squamous carcinoma; CSC, cancer stem cell; ECM, extracellular matrix; bFGF, basic fibroblast growth factor; EGF, epidermal growth factor; EMT, epithelial–mesenchymal transition.

[1] These authors contributed equally to this work.

0014-4827/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.yexcr.2012.02.038

method [11]. Therefore, CSCs may drive HNSC tumorigenesis and represent a valuable therapeutic target.

A prominent feature of CSCs is their ability to form floating spheroids in serum-free culture conditions [4]. Thus, enrichment of CSCs by sphere-forming suspension cultures has been applied successfully in several human malignancies, including HNSC [6–8,11]. This method, however, has a number of limitations. First, it is difficult to obtain pure CSCs due to proliferation of short-lived progenitor cells within the sphere cultures [12]. Second, spontaneous differentiation and cell death after stem cell divisions in sphere culture condition may occur [13]. Third, fusion of spheres occurs frequently in suspension culture, which makes it challenging to evaluate CSC traits, as evaluation is based solely on sphere number and size [14]. Finally, genetic manipulations, such as transduction of gene constructs for overexpression or knockdown, or precise evaluation of drug efficacy using entire spheroid-type CSCs, are relatively difficult due to low accessibility of gene constructs or drugs to the inner cells of spheroids compared to the outer cells of spheroids [15]. In contrast, most individual cells in adherent culture conditions are uniformly exposed to defined growth factors and oxygen tension, which allows most CSCs to maintain their stemness without spontaneous differentiation and cell death. Furthermore, the aforementioned experimental limitation in conducting gene transduction and drug efficacy tests using spheroid-type CSCs could be easily overcome by using CSCs grown in adherent culture conditions.

Extracellular matrix (ECM) of most epithelium, including head and neck tissues, is composed of laminin, collagen, fibronectin, and glycosaminoglycans, and is involved in tumor proliferation and migration [16]. A recent study reported that ECM also plays a crucial role in the maintenance of embryonic stem cell properties [17]. Furthermore, Pollard *et al.* demonstrated that pure populations of glioma stem cells can be expanded adherently in laminin-coated culture plates [13].

The aim of this study was to investigate whether HNSC-CSCs can be expanded in adherent cultures without loss of stemness traits, specifically on type IV collagen-coated culture plates.

Materials and methods

Isolation and culture of HNSC-CSCs

HNSC-CSCs were isolated from primary surgical specimens as previously described [10], and their CSC properties were validated by a number of functional assays, including self-renewal capability, stem cell marker expression, chemo-resistance, and *in vivo* tumorigenicity, as previously reported [11]. HNSC-CSCs expanded in serum-free DMEM/F12 medium supplemented with B27, N2 supplement, 10 ng/ml human recombinant basic fibroblast growth factor (bFGF; R&D Systems, Minneapolis, MN, USA), and 10 ng/ml epidermal growth factor (EGF; R&D Systems). For differentiation, HNSC-CSCs were cultured in DMEM/F12 supplemented with 10% FBS without EGF and bFGF for at least 2 weeks. Laminin, fibronectin, and type IV collagen were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Adherent cell culture with ECM proteins

For adherent cell cultures, culture plates were coated with each 10 μg/ml ECM proteins (laminin, type IV collagen, or fibronectin) for at least 3 h prior to use. Subsequently, we dissociated CSC spheres into single cells with Accutase (Sigma-Aldrich) and seeded these cells onto ECM protein-coated plates in serum-free culture medium. Every 3 days, serum-free Dulbecco's Modified Eagle's Medium Ham's F-12 (DMEM/F12) containing bFGF and EGF was replaced by fresh medium.

Quantitative reverse transcription-polymerase chain reaction (Q-RT-PCR)

Total RNA was extracted from HNSC-CSCs grown in suspension and adherent culture conditions as well as differentiated HNSC-CSCs using TRIzol (Invitrogen, Carlsbad, CA, USA). Complementary DNA was prepared using the Reverse Transcriptase Kit (Fermentas, Burlington, ON, Canada), according to the manufacturer's instructions. Quantitative reverse transcription-polymerase chain reaction (Q-RT-PCR) analysis was subsequently performed on an iCycler IQ real-time detection system (Bio-Rad, Hercules, CA, USA), using IQ Supermix with SYBR-Green (Bio-Rad). The sequences of human specific primers used were as follows:

ABCA2: F 5′-GTGTTCACCAAGATGGAGCA-3′,
R 5′-GCTTCTTGGCAAAGTTCACG-3′
ABCB1:F 5′-AGGGAAAGTGCTGCTTGATG-3′,
R 5′-GCATGTATGTTGGCCTCCTT-3′
ABCC1:F 5′-ATGAACCTGGACCCATTCAG-3′,
R 5′-CCTTCTGCACATTCATGGTC-3′
ABCC2: F 5′-AAATCCTGGTTGATGAAGGC-3′,
R 5′-GGAGATCAGCAATTTCAGCA-3′
ABCC3:F 5′-ACAACCTCATCCAGGCTACC-3′,
R 5′-GGTTGGCTGGAGAATCAAAT-3
ABCC4:F 5′-TCAGGTTGCCTATGTGCTTC-3′,
R 5′-CGGTTACATTTCCTCCTCCA-3′
ABCC5:F 5′-GGGAGCTCTCAATGGAAGAC-3′,
R 5′-CAGCTCTTCTTGCCACAGTC-3′
ABCC6:F 5′-CTGGACGAGGCTACTGCTG-3′,
R 5′-TTGTCCATGACCAGAACCC-3′
ABCG2:F 5′-CAGTACTTCAGCATTCCACG-3′
R 5′-TTTCCTGTTGCATTGAGTCC-3′

Immunocytochemistry

Adherent HNSC-CSCs were grown on coverslips in a 24-well plate at a density of 5×10^4 cells per well for 24 h, and then incubated with anti-OCT4, anti-CD44, or anti-involucrin antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA; each at 1:50 dilution) overnight at 4 °C. After washing, cells were incubated with Cy3-conjugated anti-mouse secondary antibodies. Nuclei were counterstained with 4,6-diamidino-2-phenylindole (DAPI). Images were acquired using a confocal microscope (Carl Zeiss AG, Oberkochen, Germany).

FACS analysis

HNSC-CSC sphere cells were dissociated into single cells with Accutase (Sigma-Aldrich), washed twice, and suspended in PBS. Cells were re-suspended in 100 μl binding buffer and annexin V-FITC (BD Pharmingen, San Diego, CA, USA), and then incubated at 4 °C for 30 min. After washing twice with PBS and adding 400 μl binding buffer containing 1 μl propidium iodide (PI), the

Download English Version:

https://daneshyari.com/en/article/2130660

Download Persian Version:

https://daneshyari.com/article/2130660

Daneshyari.com