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Research Article

Oral cancer/endothelial cell fusion experiences nuclear fusion and acquisition of enhanced survival potential

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ABSTRACT

Most previous studies have linked cancer–macrophage fusion with tumor progression and metastasis. However, the characteristics of hybrid cells derived from oral cancer and endothelial cells and their involvement in cancer remained unknown. Double-immunofluorescent staining and fluorescent in situ hybridization (FISH) were performed to confirm spontaneous cell fusion between eGFP-labeled human umbilical vein endothelial cells (HUVECs) and RFP-labeled SCC9, and to detect the expression of vementin and cytokeratin 18 in the hybrids. The property of chemo-resistance of such hybrids was examined by TUNEL assay. The hybrid cells in xenografted tumor were identified by FISH and GFP/RFP dual-immunofluoresence staining. We showed that SCC9 cells spontaneously fused with cocultured endothelial cells, and the resultant hybrid cells maintained the division and proliferation activity after re-plating and thawing. Such hybrids expressed markers of both parental cells and became more resistant to chemotherapeutic drug cisplatin as compared to the parental SCC9 cells. Our in vivo data indicated that the hybrid cells contributed to tumor composition by using of immunostaining and FISH analysis, even though the hybrid cells and SCC9 cells were mixed with 1:10,000, according to the FACS data. Our study suggested that the fusion events between oral cancer and endothelial cells undergo nuclear fusion and acquire a new property of drug resistance and consequently enhanced survival potential. These experimental findings provide further supportive evidence for the theory that cell fusion is involved in cancer progression.

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Introduction

Cell fusion is a dramatic and widespread cellular event in biology. Recent studies suggested that the fusion between cancer cells and host or cancer cells may provide a unifying explanation of tumor progression, such as the enhanced metastatic properties [1], epithelial–mesenchymal transition (EMT) [2], multi-drug resistance [3] and tumor heterogeneity [4]. The hybrids derived from fusion between breast cancer cells and macrophage acquired new characterizations that correlated with tumor invasion and development and overexpression of EMT-associated genes [2]. Duelli and Lazebnik demonstrated that anti-cancer drug-sensitive E1A-transformed cells and a primary fibroblast were fused, and the resulting hybrids prevented E1A-dependent apoptosis induced by etoposide (an anti-cancer drug), suggesting that the fused cells were more resistant to chemotherapeutic drugs [5].

Tumor microenvironment harbors cancer cells and stromal cells including fibroblasts, macrophages and endothelial cells (ECs) [6]. Cancer cells have many opportunities to interplay or even fuse with ECs during tumor angiogenesis and transendothelial migration. However, cancer–ECs fusion and its significance remain poorly understood as most previous studies focused mainly on cancer–macrophage fusion. Mortensen et al. showed that breast cancer cell spontaneously fused with EC in vitro and further confirmed the fusion events in vivo by both immunostaining and FISH [7]. Thus, it is intriguing to investigate whether the fusion between cancer cells and ECs lead to nuclear reprogramming, acquisition of new phenotype and changes of tumor biology behavior.

Oral squamous cell carcinoma (OSCC) is the most common malignancies occurring in oral cavity, and has tendency of increasing incidence and mortality worldwide [8]. Our previous study has found that the OSCC cells spontaneously fused with ECs in coculture system [9], and tumor necrosis factor (TNF) enhanced such fusion events. In this report, we further confirmed that the resulting hybrid cells could divide and proliferate under conventional culture condition, and express markers of both parental partners. The hybrids become more resistant to Cisplatin treatment, presenting a decrease of cell apoptosis. Xenograft experiment demonstrated that fused cells could survive and participate in tumor composition. Our results suggest that cancer/endothelial cell hybrids experience nuclear fusion and acquire enhanced survival potential of oral squamous cell carcinoma.

Materials and methods

Cell cultures

SCC-9 cell line, which was purchased from American Type Culture Collection (ATCC, Manassas, VA, U.S.), was kindly gifted by Professor Wen-feng Zhang (School and Hospital of Stomatology, Wuhan University). Primary HUVECs were kindly provided by Professor Yi-fang Zhao and Doctor Hai-xiao Zou [10]. Passages 3–8 of HUVECs were used in this study. The SCC-9 cell line and primary HUVECs were cultured in DMEM/F12 (Hyclone, UT, USA) supplemented with 10% FBS (Gibco, Carlsbad, Calif, USA) and in EC basal medium-2 (EBM-2; Lonza, Walkersville, MD) supplemented with 2% fetal bovine serum (FBS) and with EGM-2 growth factor mixture (Lonza), respectively. All cells were cultured at 37 °C in an atmosphere containing 5% CO_2.

Cell transduction and coculture

The lentiviral particles containing eGFP- and RFPpuro-vector were purchased from Genecopoeia, Guangzhou, China and GenePharma, Shanghai, China, respectively. For transduction, the lentiviral supernatants containing RFPpuro vector were added into the cultured SCC-9. After 72 h transduction, the transduced cells were selected with 0.5 μg/ml puromycin. The primary HUVECs were transduced with lentiviral supernatants containing eGFP-vector, and the transfection efficiency was confirmed by using Immunofluorescent microscopy (CarlZeiss, Germany).

EGFP-labeled HUVECs were cocultured with puromycin-resistant RFP-tagged SCC-9 with a mix of 1:1. After 16 h coculture, the cells were examined in a fluorescence microscope for the presence of dual-fluorescent positive cells.

Immunofluorescent staining

The fusion cells were positioned in a fluorescence prior to 4% paraformaldehyde fixation because the fluorescence of RFP was lost after paraformaldehyde fixation, then the fixed cells were incubated with primary antibody against vimentin (1:200, epitomic, USA) and cytokeratin 18 (1:300, CST, USA) overnight at 4 °C. Cells were then exposed to Cy3-conjugated goat anti-mouse IgG (dilution 1:200) and AMCA-conjugated goat anti-rabbit IgG (dilution 1:100) for 1 h at 37 °C. The cells were recorded with a fluorescence microscope (CarlZeiss, Germany). To examine the expression of E-cadherin of hybrid cell, the primary antibody against E-cadherin (CST, USA) was used and then exposed to the Cy3-conjugated goat anti-rabbit IgG (dilution 1:200). For detecting the fusion cells in tissue specimen, the cryosections were re-hydrated in PBS for 20 min. Then, the slides were permeablized for 10 min with 0.2% Triton-X 100 in PBS at RT and blocked with 5% goat serum for 30 min at 37 °C. The slides were incubated with primary antibody rabbit anti-GFP (1:100, Earthox, USA) or mouse anti-RFP (1:100, CWbio, China) overnight at 4 °C. After incubation with Dylight 488-conjugated goat anti-rabbit and Cy3-conjugated goat anti-mouse secondary antibodies for 45 min at 37 °C, the slides were examined by using a fluorescence microscope (CarlZeiss, Germany).

In vivo xenografts

To eliminate the protective effect of endothelial cells, we removed the endothelial cell in the coculture with puromycin and the remaining cells included hybrid cells and RFPpuro-SCC-9 cells. After expansion of the remaining cells, the mixture including puromycin-resistant hybrid cells and RFPpuro-SCC-9 cells was injected subcutaneously into the right flank of 4-week-old female nude mice at a ratio of 10,000:1 according to our FACS data. When tumor sizes reached 100 mm^2, tumors were harvested and fixed in prepared 4% paraformaldehyde for 12 h and then incubated in a sucrose series with increasing concentrations (10% for 1 h, 20% for 2 h, and 30% for 12 h).The specimens were embedded in Optimal Cutting Temperature (OCT) compound (Sakura, Labonord, France), and cut into 5 mm thick serial sections for FISH and immunostaining.