Auris Nasus Larynx 45 (2018) 566–573

Contents lists available at ScienceDirect

Auris Nasus Larynx

journal homepage: www.elsevier.com/locate/anl

Characterization of FaDu-R, a radioresistant head and neck cancer cell line, and cancer stem cells

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ARTICLE INFO

Article history:
Received 27 March 2017
Accepted 11 July 2017
Available online 24 August 2017

Keywords:
Squamous cell carcinoma
Head and neck neoplasms
Neoplastic stem cells
Radiotherapy
Radiation tolerance

ABSTRACT

Objectives: The aim of this study was to evaluate the impact of CSC on insensitivity to radiotherapy in HNSCC.
Methods: A radioresistant cell line, FaDu-R, was established using fractionated ionizing radiation. Cells with high and low CD44/ALDH activity were isolated.
Results: FaDu-R cells demonstrated significantly increased cell viability after radiation exposure compared with parental cells. $CD44^{high}/ALDH^{high}$ FaDu-R cells demonstrated significantly faster wound closure ($p < 0.05$) and more efficient invasion ($p < 0.05$) compared to the $CD44^{high}/ALDH^{high}$ FaDu cells or the $CD44^{low}/ALDH^{low}$ FaDu-R cells. There was a significant difference in tumor volume between the $CD44^{high}/ALDH^{high}$ FaDu-R cells and the $CD44^{high}/ALDH^{high}$ FaDu cells ($p < 0.05$) as well as the $CD44^{low}/ALDH^{low}$ FaDu-R cells ($p < 0.05$).
Conclusion: Cancer stem cells (CSC) were associated with invasion and tumorigenesis in a radioresistant head and neck squamous cell carcinoma (HNSCC) cell line. This concept might help to improve the understanding of these mechanisms and to develop drugs that can overcome radioresistance during radiotherapy.

1. Introduction

Head and neck squamous cell carcinoma (HNSCC) is a common malignancy that affects approximately 40,000 new patients in the United States each year [1]. To achieve a high cure rate while preserving vital structures and function, the standard treatment for locoregionally advanced HNSCC has gradually evolved from surgery to radiation-based treatment. Despite advances in therapy, which have improved quality of life, survival rates have remained static for many years. To develop more effective therapies for HNSCC, it is essential that we gain a deeper understanding of the biology of this disease and the cells that are responsible for recurrent and persistent cancer.

Accumulating evidence indicates that only a minority of cancer cells with stem cell properties are responsible for maintenance and growth of the tumor [2,3]. These cells have been designated cancer stem cells (CSC) as they have been shown to have stem cell-like qualities of self-renewal, tumorigenesis, and the ability to recapitulate a heterogeneous tumor.

Recent advances in stem cell biology have enabled the identification of CSC in solid tumors and putative stem cells in normal organs [4,5]. The solid tumor cell surface marker CD44 is currently used to identify CSC. With respect to HNSCC, Prince et al. have demonstrated that a small population of

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http://dx.doi.org/10.1016/j.anl.2017.07.011
0385-8146/

$CD44^+$ cancer cells obtained from fresh tumor tissues gave rise to new tumors in immunodeficient mice, while $CD44^-$ cancer cells did not [6].

Despite an increasing amount of research investigating the mechanisms responsible for treatment failure and resistance in HNSCC, outcomes remain largely unchanged. CSC have been shown to be especially resilient to toxic insult in a variety of malignancies and might act as critical mediators of chemo- and radio-resistance within the diverse cellular population of a tumor. CSC possess unique mechanisms to resist cell death, including modified anti-apoptotic machinery, increased pump activity, and decreased cell division [7]. In HNSCC, a higher percentage of $CD44^+$ cells in a primary tumor has been shown to be associated with higher rates of treatment failure, while cells expressing the putative CSC markers CD44, CD24, Oct4, and integrin-b1 were associated with poor outcomes following radiotherapy [8].

In this study, we evaluate the effect of a clinically relevant dose of irradiation on CSC in an isogenic model of a successively irradiated HNSCC cell line in order to determine whether CSC could be an effective targeted therapy in recurrent HNSCC after radiation therapy failure.

2. Materials and methods

2.1. Cell line

The human HNSCC cell line, FaDu, was purchased from the American Tissue-type Cell Collection (ATCC). FaDu was grown in Dulbecco's modified Eagle's medium (DMEM; GenDEPOT, Barker, TX, USA). All cell line media were supplemented with 10% fetal bovine serum (FBS; Multicell, Wisent, St. Bruno, QC, Canada) and 1% penicillin/streptomycin (Gibco, Grand Island, New York, USA) in humidified incubators at 37 °C with 5% CO_2.

2.2. Establishment of a radioresistant FaDu cell line

Exponentially growing FaDu cells were irradiated with a dose of 2 Gy × 45. Irradiation was performed with 6-MV X-rays generated by a Siemens Primus H high-energy linear accelerator (Munich, Germany). The radiation field was 10 × 10 cm, the distance from the source to target was 100 cm, and the absorbed dose rate was 200 cGy/min. The cells were subcultured between the doses of irradiation. For all assays conducted with irradiated cells, there was at least a 10-day period between the last 2-Gy irradiation and the experiment. The surviving cell line was then passaged for six months, and the radiosensitivity was determined.

2.3. Flow cytometry

FaDu and FaDu-R cultured cells were trypsinized using 0.05% trypsin and rinsed in phosphate-buffered saline (PBS). The cells were centrifuged at 800 × *g* for 5 min and resuspended in up to 1 ml serum-free medium (SFM; GenDEPOT, Barker, TX, USA). Cell suspensions were incubated with fluorescein isothiocyanate (FITC)-conjugated CD44 antibody (BD Bioscience, San Jose, CA) in the dark for 10 min at room temperature and with phycoerythrin (PE)-conjugated ALDH (Sino Biological Inc., Beijing, China). Following the reaction, the cells were rinsed with SFM and resuspended in up to 1 ml SFM. Flow analysis was performed using a fluorescence activated cell sorting (FACS) instrument (Becton–Dickinson, Mountain View, CA, USA) to sort $CD44^{high}/ALDH^{high}$ and $CD44^{low}/ALDH^{low}$ cells.

2.4. Viability assay

For the viability assay, 100 μl of cell suspension (10^4 cells/well) was dispensed in 96-well plates and pre-incubated for 24 h in an incubator (humidified atmosphere, 37 °C, 5% CO_2). Cells were irradiated at a single fraction of 8 Gy and then cultured for 72 h. Subsequently, 10 μl CCK8 (Dojindo Molecular Technologies Inc.) was added to each well and incubated for 2 h in the incubator. The absorbance was examined using a scanning multi-well spectrophotometer (Thermo Scientific).

2.5. Wound healing assay

The Radius™ 96-Well Cell Migration Assay Kit (Cell Biolabs, Inc., San Diego, CA) was used according to the manufacturer's instructions. FaDu and FaDu-R cells were seeded in the assay plate and subjected to the required treatment. Cells were then observed, and the area of the wound was measured at time 0, 24, and 48 h. Size-standardized photographs were printed on high-quality photograph paper, and the cell-free area was outlined and cut out. The percentage of invasion in initially cell-free area was determined using gravimetry.

2.6. Migration and invasion assay

The sorted cells were plated at a density of 2.5 × 10^4 cells/well onto 8-μm Transwell filters (Corning, Lowell, MA, USA) in a 24-well plate. Medium containing 10% FBS was added to the bottom wells as a chemoattractant. Twenty-four hours later, the filters were removed and then stained with 0.2% crystal violet for 15 min. The migratory cells were counted in four random fields per insert under a microscope at 20× magnification. The invasion assay was performed in a similar fashion using a BD BioCoat Matrigel Invasion Chamber (BD Bioscience), and the results are expressed as the total number of cells that invaded each filter.

2.7. Xenograft transplantation

Dissociated $CD44^{high}/ALDH^{high}$ or $CD44^{low}/ALDH^{low}$ fractions from the FaDu and FaDu-R cell lines were counted, resuspended in 50 μl Matrigel (Sigma–Aldrich), and injected subcutaneously into the right flanks of non-obese diabetic/severe combined immunodeficiency (NOD/SCID) mice using 22-gauge needles. Engrafted mice were visually inspected and palpated weekly. Mice were sacrificed using CO_2 gas inhalation when tumors attained a diameter of 1 cm post-transplantation. The tumor tissue was excised, washed in sterile PBS for