Influence of Cytoskeletal Stress Filaments on Cell Death in the Human Cervical Carcinoma

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Abstract

Epithelial tumors including cervical cancer are a leading cause of death in women. The tumors are often composed of a mixture of cell types, containing complex cellular components, which makes them a challenge to treat therapeutically. In this study, cytokeratin intermediate filaments, a type of stress filament, were investigated. These filaments are considered diagnostic of cervical cancer, and their expression is thought to protect cells from external insults that would otherwise induce cell death. Here we tested the hypothesis that cancerous cells (HeLa cells) express KRT 8/18 filaments; KRT+ cells are more resistant to induced cell death than cells lacking these filaments (KRT- cells). Both types of HeLa cells were exposed to anti-FAS antibody (CH11, 1ug/mL) to induce cell death and an inhibitor of the extracellular regulated protein kinase (MAPK) pathway (PD98059; 30μM) to evaluate the role of MAPK signaling. The KRT- cells exhibited greater sensitivity to FAS-induced death than KRT+ cells. The data support our hypothesis and suggest cytokeratin 8/18 filaments provide resistance to FAS-induced death in HeLa cells through mechanisms involving the MAPK signaling pathway. This project was supported by National Research Initiative Competitive Grant no. 2007-35303-18074 from the USDA Cooperative State Research, Education, and Extension Service.

Introduction

Cervical cancer is an ongoing health issue in women and can lead to infertility and death. It is known that 70% of all cervical cancer is caused by human papillomavirus (HPV); and typically the tumors are comprised of a mixture of cell types.

The cervical cancer cell line, HeLa cells, was established in 1951 by George Otto Gey who obtained cancerous tumor cells from the cervix of Mrs. Henrietta Lacks. The HeLa cell line is distinct from other cell lines in that the cells can be separated into two distinct populations either containing or lacking cytokeratin 8/18 intermediate filaments (KRT+ and KRT- cells, respectively).

Cytokeratin (KRT) 8/18 filaments are a type of stress filament that are thought to protect cells from programmed cell death, also known as apoptosis. The filaments reportedly decrease cytokine (death) receptor expression on the cell surface and activate intracellular anti-apoptotic signals, including cellular FLICE-like inhibitory protein (cFLIP), a labile anti-apoptotic protein [1,2] and extracellular regulated protein (cFLIP), a labile anti-apoptotic protein [1,2] and extracellular regulated protein kinase (ERK) 1/2 of the Mitogen-activated Protein Kinase (MAPK) cell survival pathway.

Below is a diagram depicting the process of apoptosis induced by the cytokine, FAS ligand. Binding of FAS ligand to the death receptor, FAS, initiates a death-inducing signaling complex (DISC), which leads to death of the cell (Figure 1). Note that activation of caspase 3 is a critical, committed enzyme in this death-inducing pathway.

The purpose of the current study was to determine whether or not KRT 8/18 filaments influence FAS-induced apoptosis in the HeLa cell.

Objectives

1) Determine influence of KRT 8/18 filaments on FAS-induced apoptosis in HeLa cells.
2) Assess importance of MAPK cell survival pathway in FAS-induced apoptosis of HeLa cells.

Materials and Methods

HeLa cells provided by the American Type Culture Collection (ATCC) were cultured 24 hours

HeLa cells previously isolated in our laboratory according to KRT profile (KRT+, >75% cells KRT 8/18 filament-containing; KRT-, <10% cells KRT 8/18 filament-containing) were cultured in flask at 37°C in 5% CO2 and 95% humidity in Eagle’s Minimum Essential Media consisting of 10% fetal bovine serum (FBS) and penicillin/streptomycin.

Verification of KRT 8/18 filament content in HeLa cells
- HeLa cells cultured in 8-well microcarrier slides until 65% confluent
- Cells fixed in 2% paraformaldehyde and post-fixed in 70% Ethanol
- Cells immunostained with anti-KRT 8/18 antibody conjugated to FITC; counterstained with DAPI
- Images obtained by fluorescent microscopy and Q-imaging color digital camera

Assessment of FAS-induced apoptosis and MAPK signaling
- Measured with CellTiter 96® AQueous One-Step Cell Proliferation Assay (MTS) (Promega); Absorbance read at 490nm using a colorimetric plate reader
- Caspase activity assay
- Measured with Caspase-Glo® 3/7 Assay (Promega); measures caspase 3/7 activity
- Luminescence (RLU) measured using a luminometric plate reader

Results

Apoptosis, as detected by MTS assay, increased in KRT-, but not KRT+ HeLa cells. There was no effect of MAPK inhibition (via PD98059) under these conditions. Staurosporine increased caspase activity in both KRT+ and KRT- cells (positive control; n=3 expts, different letters denote differences at P<0.05).

Conclusion

The present study provides initial evidence that KRT8/18 filaments in HeLa cells are cellular determinants that prevent FAS-induced apoptosis. Moreover, the protective effect of the KRT8/18 filaments is in some manner associated with the MAPK pathway.

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