Cytokine Regulation of Fas-mediated Apoptotic Signals in Human Endometrial Epithelium

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INTRODUCTION

Apoptotic cells are found in normal human endometrial epithelium\(^1\). However, the roles of the apoptotic cells in normal human endometrial epithelium are still unknown. Although various stimuli may induce apoptotic cell deaths, resulting phenotypes of apoptotic cells are very similar irrespective of apoptotic inducer. It has been reported that apoptosis-inducing cytokine receptors, such as tumor necrosis factor receptor type 1 (TNF-R type 1) or Fas antigen, are expressed on normal human endometrial epithelium\(^1\)\(^2\). It was unknown whether these apoptosis receptors really transduct any apoptotic signals in endometrial cells until we have reported a receptor-specific apoptotic signal in a human endometrial cell line\(^3\). Yet, there is still no report on analysis on regulation of apoptotic signals in human endometrium. We have analyzed cytokine regulations of apoptotic signals in endometrium using a differentiated endometrial adenocarcinoma cell line which is sensitive to a Fas-mediated apoptotic stimulus, since proliferation and differentiation of endometrial cells are regulated not only by endocrinological mechanisms but also by autocrine or paracrine mechanisms of intrauterine cytokine network. In the present study, we show that some cytokines can regulate sensitivity to the apoptotic signals, and that endometrial apoptosis may be regulated by intrauterine cytokine network.

MATERIALS & METHODS

Cell Lines and Culture: All cell lines were cultured in Opti-MEM medium (GIBCO-BRL) containing 5% bovine calf serum, 50 IU/l of streptomycin (GIBCO-BRL) and 50 IU/L penicillin (GIBCO-BRL).

Northern Blot Analysis: Our protocol of Northern blotting was described in details elsewhere\(^4\).

Flowcytometric Analysis: Staining procedure for cell lines was described before\(^5\).

Immunostaining of the Cell Lines and Frozen Section of the Normal Endometrial Tissues: Immunostaining procedure was described elsewhere\(^6\).

Cell Proliferation Assay: Non-radioisotope cell proliferation assay kit, XTT (Boehringer-Mannheim), was used for this study. Each assay was tried more than 3 times to reach a reproducible result.
DNA Fragmentation Assay: Genomic DNAs were extracted from anti-Fas-treated cells in a usual manner and digested with RNase A for 2 hours at 56 °C. Ten μg of the purified genomic DNAs were electrophoresed onto 1% agarose gel, and stained with ethidium bromide.

RESULTS

First, a cell line which was sensitive to Fas-mediated apoptosis was selected from 3 differentiated endometrial adenocarcinoma cell lines, HEC-1, HHUA, Ishikawa. Northern blot analysis and flowcytometric analysis revealed Fas-antigen expression on these 3 cells. Cell proliferation of HHUA line was dose-dependently suppressed by anti-Fas IgM antibody. In HHUA cells, apoptotic bodies appeared within 2 hours after anti-Fas treatment, which was accompanied by DNA fragmentation determined by agarose gel electrophoresis.

In order to examine whether any cytokine interfere apoptotic signal in human endometrial cells, HHUA cells were stimulated with anti-Fas IgM two days after they were pretreated with one of 11 cytokines which included IFN-α, IFN-γ, EGF, and so on. Both IFN-γ and EGF raised the sensitivity levels of the endometrial cell lines to the apoptotic signal, while they had no effect on Fas antigen expression level in cell surface of the cytokine-stimulated cells. HHUA cells, which were co-cultured with normal human endometrial stromal cell layer, revealed resistance to the anti-Fas-induced apoptosis.

DISCUSSION

Pretreatment with a low concentration of cytokine interfered the Fas-mediated apoptotic signal, which indicates that apoptotic signals in endometrium is regulated by intrauterine cytokine network. IFN-α/β and EGF served to raise the endometrial cells’ sensitivity level to the apoptotic signal. However, these three cytokines showed no effect on Fas antigen expression on the cell surface. This means that the increased sensitivity was caused by strengthened apoptotic signal but not by overexpression of Fas receptor.

Interestingly, the pretreatment of the endometrial cells with EGF affected anti-Fas IgM-treated HHUA cells differently from the pretreatment with TGF-α did. This means that sensitivity to apoptosis in human endometrial epithelium may be regulated by some tyrosine-phosphorylated proteins which were activated according to the degree of bindness of erbB receptors with certain cytokines; the degree and the kind of cytokines are varied from receptors to receptors7).

Endometrial stromal cells secrete various kinds of cytokines which regulate differentiation and proliferation of endometrial epithelial cells. In this study, Fas-mediated apoptosis of HHUA was inhibited by co-culturing it with stromal cells. These results taken together, it is highly possible that endometrial stromal cell also play a key role in regulating endometrial apoptosis.

REFERENCES