Differential Expression of HDAC1 and HDAC2 in Human Endometrial and Endometriotic Cells

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Although the existence of endometriosis, a painful, enigmatic disease, has been known for years, our current knowledge of its pathophysiology and pathogenesis remains unclear. There are various theories that try to explain the mechanism whereby endometrial cells are able to grow in the peritoneum and other ectopic sites, forming lesions that are hormone–dependent. Previous studies support the role of genetic and epigenetic mechanisms in endometriosis. HDACs play an important role in gene suppression and regulation of genes involved in apoptosis, cell cycle, invasion and proliferation. Our study was designed to measure the basal and steroid hormone regulation of the expression of HDAC1 and HDAC2 in normal endometrial and endometriotic cells. This study will help clarify the role of HDACs in endometriosis.

Objective: To determine the basal expression of histone deacetylases (HDAC) 1 and 2 in human endometrial and endometriotic cells and their regulation by steroid hormones.

Design: Human endometrial cells and endometriotic cell lines, cultured in vitro were treated with physiological levels of steroid hormones (progesterone and estradiol). The levels of mRNA expression were compared with untreated (vehicle only) cells.

Setting: Ponce School of Medicine Research Laboratory

Main Outcome Measures: To measure levels of HDAC1 and HDAC2 gene expression in human endometrial and endometriotic cells, we used TaqMan Gene Expression Assays. We determined the pattern of gene expression regulated by steroid hormones by incubation with estradiol and progesterone and measuring gene expression levels as compared to controls.

Background

Endometriosis is a common medical condition characterized by growth beyond or outside the uterus of tissue resembling endometrium, the tissue that normally lines the uterus. Histone deacetylases (HDACs) are enzymes that modify key residues in histones to regulate chromatin structure and act as transcriptional repressors of genes. Based on their role in cell cycling, apoptosis and differentiation, HDACs have been chosen as therapeutic targets for the treatment of cancer and other diseases. HDACs are divided into different classes based on sequence conservation: HDACs 1, 2, 3 and 8, which belong to class 1 and HDACs 4, 5, 6, 7 and 9 which belong to class 2. We focused our study in class 1, specifically HDACs 1 and 2 because they play an important role in steroid-hormone dependent gene expression.

Specific Aims

Through our research, we wanted to achieve two specific objectives:

1) To determine the basal expression of histone deacetylases (HDAC) 1 and 2 in human endometrial and endometriotic cells. We hypothesized that HDAC1 and HDAC2 genes are highly expressed in endometriotic cells compared to normal endometrial cells.
2) To determine if steroid hormones regulate the expression of histone deacetylases (HDAC) 1 and 2 in human endometrial cells. We hypothesized that estradiol and progesterone increase the expression of HDAC1 and HDAC2 genes in endometrial cells.

Methods

Normal endometrial (HESC, HES) cells were cultured as previously described in phenol-red free Dulbecco's Modified Eagle's Medium Nutrient (DMEM) supplemented with 10% FBS(charcoal/dextran-treated FBS), 1% ITS (insulin, transferring, selenious acid), puromycin (500 ng/mL) and 1% ampicillin/streptomycin. The endometriotic cell line (ECL) was cultured in ATCC complete growth media plus fetal bovine serum to a final concentration of 10%. Hs578T (breast cancer cell) were cultured in ATCC-formulated Dulbecco's Modified Eagle's Medium and the following components were added to the medium: 0.01 mg/mL bovine insulin; fetal bovine serum to a final concentration of 10%. For these experiments, cells were cultured in 6-well tissue culture dishes or 100-mm tissue dishes until 90% confluence at which time medium was replaced by fresh serum-depleted medium (1% of FBS). After 24-hour growth, cells were incubated with 10^{-8} M estradiol (E2) or 10^{-7} M of progesterone (P4) or E2 + P4 in 1% serum-free media. Control cells received vehicle (0.1% ethanol DMEM). After 24 hours, total RNA was isolated using the RNAeasy kit (Qiagen, Valencia, CA) following standard procedures. DNA (cDNA) (10 ng) was subjected to 50 cycles of qPCR in an iCycler (Bio-Rad) according to the manufacturer's instructions. Gene expression levels were determined using TaqMan®Gene Expression Assays (ABI) for each target gene.

RESULTS

We detected basal gene expression of HDAC1 in all the cell lines under study. The endometriotic cell line had the highest expression of HDAC1 in comparison with both normal endometrial cells (HES, HESC) and BC cells (~10 fold higher). The difference in expression in HES and HESC compared to ECL was statistically significant. (P=.05 + .04 + .06, respectively).

The endometriotic cell line had the highest expression of HDAC2 in comparison with both normal endometrial cells (HES, HESC) and BC cells. There were significant differences in the levels of expression of HDAC2 in ECL vs BC, HES and HESC. (P=.01 +.00 +.03, respectively).

E2 did not increase the expression of HDAC1, but was able to increase the expression of HDAC2 over 20-fold compared to untreated controls. P4 increased the expression of HDAC1 2-fold, while it dramatically upregulated the expression of HDAC2 (~50-fold). E2 + P4 treatment upregulated the expression of both genes (~2–3 fold). E2 was able to antagonize the effects of P4 on the expression of HDAC2.

HDAC1 was not regulated by the steroid hormone treatment in endometrial epithelial cells while HDAC2 expression was upregulated 2-fold by P4.

CONCLUSIONS

We determined the expression of HDAC1 and HDAC2 in endometriotic and endometrial cell lines. The expression of both HDAC1 and HDAC2 genes is significantly higher in the disease cells (ECL) as compared to normal endometrial cells (HES and HESC). Steroid hormone treatment induced an upregulation of HDAC1 and HDAC2 in endometrial stromal cells. HDAC1 expression was increased by P4. HDAC2 expression was increased by both E2 and P4.These preliminary studies indicate that HDAC1 and HDAC2 may play an important role in endometriosis by modulating the expression of genes, which results in this complex and enigmatic disease. The role of steroid hormones in modulating the expression of these genes deserves further characterization.

ACKNOWLEDGMENTS

The author thanks the Endometriosis Research Program staff for their support and help during this project.

RESOURCES

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