Gonadotropin-releasing hormone and gonadotropin-releasing hormone receptor are expressed at tubal ectopic pregnancy implantation sites

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Objective: To investigate whether gonadotropin-releasing hormone (GnRH) and GnRH receptor (GnRHR) are expressed at tubal ectopic pregnancy sites, and to study the potential role of GnRH signaling in regulating immortalized human trophoblast cell viability.
Design: Immunohistochemical and experimental studies.
Setting: Academic research laboratory.
Patient(s): Fallopian tube implantation sites (n = 25) were collected from women with ectopic pregnancy. First-trimester human placenta biopsies (n = 5) were obtained from elective terminations of pregnancy.
Intervention(s): None.
Main Outcome Measure(s): GnRH and GnRHR expression was examined by means of immunohistochemistry and histoscoring. Trophoblastic BeWo choriocarcinoma and immortalized extravillous trophoblast (HTR-8/SVneo) cell viability was examined by means of cell counting after incubation with GnRH and/or GnRH antagonist (Antide).
Result(s): GnRH and GnRHR immunoreactivity was detected in cytotrophoblast, syncytiotrophoblast, and extravillous trophoblast in all women with tubal pregnancy. GnRH immunoreactivity was higher and GnRHR immunoreactivity lower in syncytiotrophoblast compared with cytotrophoblast. GnRH and GnRHR immunoreactivity was detected in adjacent fallopian tube epithelium. Whereas neither GnRH nor Antide altered HTR-8/SVneo cell viability, treatment with GnRH significantly increased the overall cell viability of BeWo cells at 48 and 72 hours, and these effects were abolished by pretreatment with Antide.
Conclusion(s): GnRH and GnRHR are expressed in trophoblast cell populations and fallopian tube epithelium at tubal ectopic pregnancy sites. GnRH increases BeWo cell viability, an effect mediated by the GnRHR. Further work is required to investigate the potential role of GnRH signaling in ectopic pregnancy. (Fertil Steril® 2016;105:1620–7. ©2016 by American Society for Reproductive Medicine.)
Key Words: GnRH, GnRHR, ectopic pregnancy, trophoblast, fallopian tube

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An ectopic pregnancy is a pregnancy-related complication that is characterized by aberrant embryo implantation outside the normal endometrial cavity, with 95% occurring within the fallopian tube (1). Ectopic pregnancies occur in 1%–2% of all pregnancies (2), and, in the Western World, they remain the most common cause of maternal mortality in the first trimester of pregnancy (3). The expressions of several genes are known to be altered in the fallopian tubes of women with tubal ectopic pregnancies (4). In addition, some trophoblast-derived factors have been identified that may contribute to tubal implantation and placentation. For example, leukemia inhibitory factor is expressed in trophoblasts from ectopic pregnancies and supports trophoblast adhesion to a fallopian tube cell line (5). Moreover, trophoblast-secreted factors up-regulate galactin-1, a molecule involved in intrauterine

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implantation, in fallopian tube epithelial cells in vitro (6). Further work characterizing gene expression at the fallopian tube implantation site is important to improve understanding of the etiology of ectopic pregnancy.

Gonadotropin-releasing hormone (GnRH) and its G-protein–coupled receptor (GnRHR) play a central role in regulating reproductive function (7, 8). Best known for their expression and function in the central nervous system, GnRH and GnRHR have also been detected in a variety of other normal and neoplastic tissues, both within and outside the reproductive system (9, 10). Indeed, GnRH and GnRHR have been detected in both the maternal and fetal components of first-trimester human placenta (11, 12). On the fetal side, GnRH and GnRHR are expressed in cytotrophoblast, syncytiotrophoblast, and extravillous trophoblast cells (13, 14). On the maternal side, GnRH and GnRHR mRNA and protein are expressed in the decidua (15). Additionally, GnRHR has been detected in rat oviduct during the postimplantation period (16). However, whether or not GnRH and GnRHR are expressed in tubal ectopic pregnancy is unknown.

In first-trimester human placenta, GnRH has been shown to stimulate hCG secretion in placental explants, primary trophoblasts, and choriocarcimoma BeWo cells (17–19). Moreover, GnRH has been shown to increase primary and HTR-8/SVneo immortalized extravillous trophoblast cell invasion (13,20–22). These biologic functions contribute significantly to the establishment of early pregnancy, but the role of GnRH signaling in ectopic pregnancy has not been studied.

In the present study, we examined the expression of GnRH and GnRHR in fallopian tube implantation sites from women with ectopic pregnancy and the effect of GnRH and a GnRH antagonist on cell viability in two immortalized trophoblast cell lines.

MATERIALS AND METHODS

Reagents and Antibodies

Native human GnRH I and the GnRH antagonist, Antide, were purchased from Bachem (Belmont, CA). Rabbit polyclonal antibodies against mouse GnRH (Ab5617; antigen sequence identical to human GnRH) and β-hCG (Ab9376) were purchased from Abcam. Mouse monoclonal antibody against human GnRHR (clone F1G4) was purchased from Thermo Scientific. Mouse monoclonal antibody against human Ki67 (clone MIB-1) was purchased from Dako. Mouse monoclonal human leukocyte antigen G (HLA-G) antibody (clone 4H84) was obtained from Exbio. Mouse monoclonal antibody against human cytokeratin-7 (clone OV-TL 12/30) was purchased from Millipore. Normal rabbit control IgG (sc-2027) and mouse control IgG1 (M5284; clone MOPC21) were purchased from Santa Cruz Biotechnology and Sigma-Aldrich, respectively. Trypan Blue solution (0.4% in phosphate-buffered saline solution [PBS]) was purchased from Life Technologies.

Tissue Collection

This study was approved by the Research Ethics Board of the University of British Columbia (H07-01149) as well as the Scotland A Research Ethics Committee (LREC 04/S1103/20), and every patient provided informed written consent. Fallopian tube implantation sites (n = 25) were collected from women undergoing salpingectomy for the treatment of tubal ectopic pregnancy. First-trimester placenta biopsies (6–12 weeks, n = 5) were obtained from women undergoing elective termination of pregnancy.

Immunohistochemistry and Histoscore Analysis

Fallopian tube samples containing ectopic implantation sites and first-trimester human placenta samples were fixed in 4% formaldehyde and embedded in paraffin for sectioning. Sections were deparaffinized in xylene, rehydrated through graded ethanol, and processed for wet heat-induced antigen retrieval in a steamer for 20 minutes with a modified citrate buffer (pH 6.1; Dako). Sections were incubated in 3% H_2O_2 in PBS for 30 minutes at room temperature to quench endogenous peroxidase, and then blocked with serum-free protein block for 1 hour at room temperature. Sections were incubated with antibodies against GnRH (20 μg/mL), GnRHR (20 μg/mL), Ki67 (4 μg/mL), cytokeratin-7 (5 μg/mL), HLA-G (5 μg/mL), and β-hCG (5 μg/mL) overnight at 4°C. Immunoreactivity was detected with the use of the horseradish peroxidase–linked Envision system (Dako) and 3,3′-diaminobenzidine chromogen solution. Exposure time to 3,3′-diaminobenzidine chromogen solution for all slides was 5 minutes. Slides were counterstained with Harris hematoxylin (Sigma-Aldrich) for 2 minutes, dehydrated through graded ethanol to xylene, mounted in a xylene-based mounting medium, and observed under a light microscope (Leica).

Immunohistochemical scoring (histoscore) was performed as previously described (23, 24) with minor modifications. Briefly, five representative fields containing placental tissue and five representative fields containing fallopian tube were examined per patient (n = 25) with the use of a Zeiss light microscope at ×200 magnification. The intensity of GnRH and GnRHR immunostaining was classified into four categories (0 = negative; 1 = weak; 2 = moderate; and 3 = strong). Immunostaining was scored in five cell populations; villous cytotrophoblast, syncytiotrophoblast, extravillous trophoblast, fallopian tube epithelium, and fallopian tube stroma. The percentage of cells in each cell population with negative, weak, moderate, or strong staining was noted. A histoscore for each cell population in each field was calculated as follows: histoscore = 0 × percentage of negative staining cells + 1 × percentage of weak staining cells + 2 × percentage of moderate staining cells + 3 × percentage of strong staining cells. The histoscore of each sample was calculated as the mean of the histoscores from five different fields.

Cell Culture

The HTR-8/SVneo immortalized extravillous trophoblast cell line was a kind gift from Dr. P. K. Lala (Western University, London, Onatrio) and was cultured in Dulbecco Modified Eagle Medium (DMEM; Life Technologies) supplemented with 10% fetal bovine serum (FBS) and antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin; Life Technologies). The expression of GnRHR in HTR-8/SVneo cells has been previously