

GROWTH HORMONE-RELEASING HORMONE (GHRH) ANTAGONIST INDUCES APOPTOSIS IN HUMAN DECIDUAL STROMAL CELLS

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Purpose: Decidualization of the endometrium occurs in human pregnancy with the implantation of the embryo. Decidualization in humans affects all uterine compartments, including endometrial stromal cells. Endometrial stromal cells are mesenchymal cells that play an important role on decidual programming of human pregnancy. Growth hormone-releasing hormone (GHRH) is secreted by the hypothalamus and stimulates the synthesis and secretion of GH from the pituitary. The expression of GHRH and its receptors has been demonstrated in peripheral tissues. In recent years, many antagonistic analogs of GHRH have been developed. In the present study, we examined the mechanisms of GHRH antagonist-induced apoptosis of human endometrial stromal cells and the effects of GHRH antagonist on the elevation of MAPKs and GADD45 α expression, indicating the role of GHRH antagonists in embryo implantation and early pregnancy.

Methods and Materias: Human decidual stromal cells were isolated from the decidual tissue from healthy women undergoing elective pregnancy termination of a normal pregnancy at 6- to 12-wk gestation, after informed consent. GHRH antagonist JMR-132 was synthesized by solid phase methods. RT-nested PCR was applied for detection of GHRH and SV1 of the GHRH-R. Immunoblot analysis was performed to investigate the effects of GHRH antagonists in the activation of ERK1/2, JNK, caspase-3, caspase-9 and GADD45 α . ERK1/2 inhibitor (U0126) and JNK inhibitor (SP600125) were pretreated for 30 min to evaluate the effects of MAPKs. Human GADD45 α siRNA was used to knock down the expression of GADD45 α . TUNEL assay was performed to detect apoptotic cells. Cell viability was estimated by [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide] (MTT) assay.

Results: SV1 of GHRH-R mRNA was detected in decidual stromal cells (13 out of 22, 59%). GHRH mRNA was detected in 6 out of 22 (27%) decidual stromal cells. The GHRH antagonist decreased cell viability in a dose- and time-dependent manner. Apoptosis was induced with increased TUNEL apoptotic cells and cleaved caspase-3 activity after GHRH antagonist treatment. GHRH antagonist activated ERK1/2 and JNK, and the activation was abolished by U0126 and SP600125. Cells pretreated with U0126 and SP600125 were rescued from GHRH antagonist-mediated cell growth inhibition. Moreover, U0126 and SP600125 abolished GHRH antagonist-induced apoptosis and downstream GADD45 α signaling. GHRH antagonist-mediated cell growth inhibition was rescued by knockdown of endogenous GADD45 α with siRNA. Cells treated with MAPK inhibitors or GADD45 α siRNA were refractory to GHRH antagonist-induced apoptosis.

Conclusion: Our study demonstrates that the GHRH antagonist suppresses the growth of decidual stromal cells by inducing apoptosis through the activation of ERK1/2 and JNK-dependent induction of GADD45 α signaling. Our findings represent a new concept regarding the mechanisms of GHRH antagonist-induced inhibition of cell viability and apoptotic effects in human decidual stromal cells, suggesting that GHRH antagonists have strong effects on decidual programming of human pregnancy.