

Elucidation of signaling pathways of GnRH II in extravillous cytotrophoblasts**Chou C.S, MacCalman C.D, Leung C.K.**

Department of Obstetrics and Gynecology, University of British Columbia, Vancouver, BC, Canada.

INTRODUCTION: Gonadotropin-Releasing Hormone (GnRH-I) and a novel form of Gonadotropin-Releasing Hormone (GnRH-II), which are expressed in extravillous cytotrophoblasts (EVT), have been shown to mediate certain functions in the human placenta. To date, the signaling pathways of GnRH II in the human placenta remain poorly characterized. In view of these observations, we have elucidated these pathways by screening different vectors, which contain specific cis-acting DNA binding sequences for various transcription factors. Our strategy provides a novel approach for profiling signal transductions of GnRH II.

DESIGN: Prospective, controlled study.

MATERIALS AND METHODS: Samples of first trimester placenta tissue were obtained from women undergoing elective termination of pregnancy. Chorionic villi explants were minced and washed with PBS and then plated these explants in culture flasks in DMEM supplemented with 10% FBS. The cells were separated from the explant tissue by a brief trypsinization and subcultured between 10-17 days from initial plating. The purity of the cultures was determined by immunostaining for vimentin and cytokeratin. The trophoblastic cells were washed with PBS and cultured in serum free DMEM for a further 4 hours. The expression plasmids encoding enhancer elements of transcription factor binding sites on the upstream of the luciferase gene were constructed and transiently transfected into EVT using Lipofectamine. Forty hours after transfection, the cells were left untreated as a control or treated with 1nM GnRH II for 6 h. Cells were then harvested using cell lysis buffer. Luciferase expression assay was conducted and normalized by β -galactosidase values.

RESULTS: Among 14 constructs transfected, GnRH II in EVT only activated the Activator Protein-1 (AP-1), cAMP Response Element (CRE) and p53 pathways. GnRH II induced AP-1, CREB (CRE binding protein) and p53 activity by 2.4, 3.2 and 1.8 folds respectively. Furthermore, AP-1, CRE and p53 activity responded GnRH II in a dose and time dependent manner.

CONCLUSION: GnRH II exerted its effects on EVT via AP-1, CRE and p53 signal transduction pathways.

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