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Identification of the Relaxin-Responsive Cells in the Human Choriondecidua at Term

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Abstract

The decidua of the human maternal-fetal interface is a local source of intrauterine relaxin and the choriondecidua expresses its receptor (LGR7). Since these tissues consist of a variety of cells, we sought to identify the primary cell(s) responsible for LGR7 expression and relaxin responsiveness.

Keywords

Relaxin; LGR7; chorion; decidua; cAMP; primary cells

Introduction

The human fetal membranes lie between the myometrium and amniotic cavity, forming the fetal container or gestational sac. This complex tissue, together with the decidua, forms a large, immunologically-unique maternal-fetal junction. Autocrine-paracrine signaling across this interface is essential for both pregnancy maintenance and the initiation of parturition,¹ although the details of this crosstalk are not yet fully known. Human H2 relaxin is a systemic hormone from the corpus luteum of pregnancy, but is also secreted locally by the decidua.¹ It has been shown that the choriondecidua expresses its G-protein-coupled receptor (LGR7),² and upon receptor binding, relaxin increases matrix metalloproteinases.^{3,4} While collagen remodeling is necessary for growth, its overactivity leads to the preterm premature rupture of the membranes and preterm birth.⁵ Since the choriondecidual tissue consists of a variety of maternal and fetal cells, we sought to identify the primary cell(s) responsible for LGR7 expression and relaxin responsiveness.

Materials and Methods

Fetal membranes were obtained with IRB approval from women undergoing term Cesarean sections before labor. After removing the amnion, the decidua was scraped from the chorion before enzymatic digestion (0.2% Collagenase I, 0.2% DNaseI and 0.125% trypsin, 0.02% CollagenaseA, 0.02% DNaseI, respectively). Chorion cells and decidual stromal cells were isolated using a Percoll density gradient separation method, collecting at the 1.049–1.062g/ml interface and 1.0147–1.045g/ml interface, respectively. Decidual macrophages were enriched with CD14 immunomagnetic positive selection (StemCell Technologies) before sorting on a fluorescence activated cell sorter. Following isolation, cells were cultured in RPMI 1640 or DMEM/F12 supplemented with 10% FBS and antibiotics at 37°C under 5% CO₂ 95% air. Decidual stromal cells were further decidualized with MPA (1uM), E2

(10nM) and PGE₂ (1uM) for 4 days. Decidualization was confirmed by significantly increased ($p=0.03$) prolactin gene expression. THP-1 cells and phorbol 12-myristate 13-acetate (PMA)-activated THP-1 cells were used as controls for primary decidual macrophages. RNA was isolated using RNeasy Mini or Micro Kits (Qiagen), and LGR7 gene expression was determined with TaqMan real-time PCR (ABI), with results normalized to 18S expression in each sample. Following relaxin treatment for 30 minutes, intracellular cAMP was measured with the cAMP Biotrak Enzyme Immunoassay (Amersham), and results were normalized to total protein in each sample as determined by DC Lowry protein assay (BioRad). Statistical significance was assessed by the Kruskal-Wallis Test (non-parametric ANOVA).

Results and Discussion

Real-time PCR showed that decidual stromal cell LGR7 gene expression was significantly increased ($p=0.03$) following decidualization with MPA (1uM), E₂ (10nM) and PGE₂ (1uM) for 4 days. However, after decidualization, the significant cAMP response to added relaxin was lost, while non-decidualized cells retained a significant response at 10nM relaxin ($p<0.05$ compared to controls). This suggests there may be an alternative pathway for relaxin action in decidualized stromal cells. Decidual macrophages ($n=7$ from different patients) expressed low levels of LGR7 mRNA relative to THP-1 cells, correlating with their small cAMP dose-response to added relaxin. In comparison, THP-1 cells had a robust cAMP response and treatment with 1 and 10nM relaxin caused significant increases in cAMP ($p<0.01$, $p<0.05$, respectively). However, THP-1 cells activated with PMA expressed significantly less LGR7 than before treatment ($p<0.05$ compared to untreated THP-1), corresponding with their lower cAMP response. LGR7 gene expression in primary chorionic fibroblasts was high, consistent with their strong cAMP dose-response to relaxin, which showed significant increases ($p<0.05$) in cAMP at 1 and 10nM relaxin compared to untreated controls.

Conclusions

The chorionic cells are the most relaxin-responsive cells identified to date in the human fetal membranes and are being used for further characterization. The decidual stromal cells expressed low levels of LGR7 mRNA which was increased after decidualization. However, this treatment reduced their cAMP response, suggesting that relaxin may be signaling through an alternate pathway in this situation. Clearly, LGR7 protein and splice variant expression warrant investigation in these cells and may partially explain these results.

Acknowledgments

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