ISP-7-1 Relaxin has anti-apoptotic effects on human trophoblast-derived HTR-8/SV neo cells

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[Objective] The present study was conducted to evaluate the effects of human relaxin on apoptosis in the human trophoblast derived HTR-8/SV neo cell line, which is a possible model of human extravillous trophoblasts (EVTs). [Methods] HTR-8/SV neo cells, cultured in phenol red free RPMI1640 medium, were treated with different doses of human recombinant (rH2) relaxin in serum-deprived conditions. RT-PCR was used for evaluating relaxin receptor : LGR7 expression in HTR-8/SV neo cells. The cell viability was determined by MTS assay. Cell death was examined by TUNEL assay. Furthermore, we investigated caspase-3, cleaved PARP, and Bcl-2 expressions by Western blot analysis to recognize the translational effects of anti-apoptotic and pro-apoptotic proteins.[Results] LGR7 mRNA expression was observed in HTR-8/SV neo cells. Compared with untreated control cultures, treatment with rH2 relaxin increased the number of viable cells, but decreased the TUNEL-positive rate in HTR-8/SV neo cells. Western blot analysis revealed that treatment with rH2 relaxin decreased the expression of caspase-3 and cleaved PARP, but in contrast increased Bcl-2 expression in those cells. [Conclusion] These results suggest that rH2 relaxin has anti-apoptotic effects on HTR8/SV neo cells by decreasing pro-apoptotic caspase-3 and cleaved PARP expression and up-regulating anti-apoptotic Bcl-2 expression.

ISP-7-2 Remarkable modulation of the Golgi and trans-Golgi network in fused BeWo cells

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[Objective]]Formation of syncytia from mononuclear cells leads to a number of changes in cellular physiology and function. We hypothesized that such changes will be reflected at the organelle level. Herein, we focus on alterations to the Golgi complex (GC) and trans-Golgi network (TGN) associated with BeWo cell fusion.[Methods] Cultured BeWo cells were used as surrogates for cytotrophoblasts and were induced to fuse by treatment with forskolin (FK). BeWo cells treated with FK for 24, 48, and 72 hours were compared to untreated control cells using immunofluorescence (IF) and western blotting (WB). [Results] In control cells, the GC and TGN markers had a perinuclear distribution as determined by IF microscopy. In contrast, fused cells had remarkablly altered GC and TGN architecture, which were typically formed giant structures. The immunolabeling patterns suggested that the GC and TGN had increased in amount when compared to control. This was investigated using WB ; a time-dependent increase in GC markers proteins was found in fused cells.[Conclusion] We show that there is a remarkable alteration in the architecture of GC and TGN along with an increase in GC and TGN marker proteins in fused cells. We propose that these results may have implications for alteration in protein synthesis, subsequent processing in the process of syncytialisation.

ISP-7-3 The regulation of chemokine production involving protease-activated protein-1 in human granulosa cells

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[Objective] Protease-activated receptor (PAR) is a peptide receptor that carries its own ligand. The ligand remains hidden until it is revealed by selective cleavage of PAR's amino-terminal exodomain. In order to investigate the role of PAR-1 in human ovulation, we studied the production of interleukin (IL)-8, via PAR-1 in human granulosa cells.[Methods] KGN cells were cultured and incubated with TRAP-6, PAR-1 antagonist (PPACK), p38 inhibitor (SB203580), PLC inhibitor (U73122) and/or PKC inhibitor (GF109203X). IL-8 were measured by ELISA. The phosphorylations of p38 induced by TRAP-6 were analyzed by western immunoblot analysis. This study was approved by the institutional review board.[Results] TRAP-6, a PAR-1 activator, increased the production of IL-8 ($p<0.001$). The TRAP-6-stimulated IL-8 production was inhibited by SB203580, U73122 or GF109203X ($p<0.001$). The p38 activities were induced by TRAP-6, which was also suppressed by SB 203580, U73122 or GF109203X.[Conclusion] Our data indicated that IL-8 levels were regulated by TRAP-6 by a mechanism involving a MAP kinase system in human GC. These results suggest that PAR-1 may play an important role in human ovulatory processes by involving IL-8-induces leukocyte chemotaxis and activation.