

ROLE OF APOPTOTIC PROTEINS IN INTERCELLULAR FUSION AND DIFFERENTIATION IN BeWo, A CYTOTROPHOBLAST MODEL.

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Intercellular fusion from mononuclear villous cytotrophoblastic precursor cells into a multinuclear syncytiotrophoblast is the normal route of differentiation in the human placenta. The process is accompanied by changes in expression of apoptosis-related proteins; the specific nature and timing of which are unclear. Others have suggested that cleaved caspase-8 may participate in control of trophoblast intercellular fusion. Using the choriocarcinoma BeWo, a model for villous cytotrophoblast differentiation, we studied the role of apoptosis-related proteins in the fusion process. We quantified levels of protein by Western blot and mRNA by RT-PCR for Bcl-2, Bcl-xL, Bax, p53, caspase-3 (CASP3), and caspase-8 (CASP8). Forskolin treatment of BeWo cells for 0, 24, 48, and 72 h resulted in progressive differentiation and intercellular fusion; by 72 h 80% of nuclei were contained in syncytia, compared to 10% in controls without forskolin. Forskolin induced increased Bcl-2 protein ($P<0.01$) and mRNA ($P<0.05$), with a concurrent increased resistance to cisplatin-induced apoptosis. Bax and p53 proteins ($P=0.02$, $P<0.002$, respectively) and mRNA ($P<0.04$, $P<0.01$, respectively) decreased. Levels of CASP3 ($P<0.0005$) and CASP8 ($P=0.0001$) protein decreased during differentiation, which correlated with decreased transcription ($P<0.05$, $P<0.0001$, respectively), rather than caspase activation. We studied further the role of CASP8 in a BeWo subline (C8-4) in which CASP8 was silenced (>94% reduction in protein) using stable transfection with siRNA targeted to CASP8. Forskolin-induced fusion was delayed ($P<0.05$) by 24 h in C8-4 (peak fusion at 96 h) compared with controls (peak fusion at 72 h). Thus, we surmise that differentiation and intercellular fusion of BeWo, a trophoblast model, results in a relatively apoptosis-resistant state by increased Bcl-2 and diminished pro-apoptotic protein expression. We further predict that procaspase-8 contributes to the control of trophoblast differentiation.