—Short Review—

Dramatic Alterations in the Protein Biosynthetic Machinery Accompany Trophoblast Fusion

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Abstract

This report represents a summary of a seminar presentation at Nippon Medical School on September 24, 2012. It is a synopsis of some of our research on trophoblast fusion.
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Key words: trophoblast, BeWo cells, cell fusion, biosynthesis

We developed methods for the preparation of an enriched fraction of the apical plasma membrane of the syncytiotrophoblast (STB) of the human placenta to carry out a proteomics analysis of this membrane. We identified 350 proteins having two or more unique peptides by mass spectrometry. Our initial focus has been on dysferlin (DYSF) and myoferlin (MYOF)[1,2]. At the time we discovered these proteins in the STB they were only known in skeletal muscle.

In the human placenta, DYSF resides in the apical plasma membrane of the STB and was not detected in cytotrophoblasts (CTB), the progenitor cells of the STB. When primary CTBs were isolated from term placentas and maintained in cell culture they spontaneously fuse after a few days; following cell fusion, DYSF was expressed[1]. We have also used BeWo cells, a trophoblastic cell line that can be induced to fuse following treatment with forskolin. BeWo cells behaved in a manner similar to primary CTBs in that they express DYSF following cell fusion[2]. Thus BeWo cells recapitulate the situation in CTBs.

While this report does not focus on DYSF and MYOF, finding these proteins in the human placenta led to the other studies that form the basis of this report. Using immunofluorescence microscopy (IFM) to monitor cell fusion and DYSF expression, we noticed that a portion of the DYSF was in unusually shaped structures with a perinuclear distribution in fused BeWo cells. We hypothesized that this labeling pattern represented Golgi complex-associated DYSF. To test this we did double-label IFM with antibodies to DYSF and Golgi-marker proteins. This hypothesis was not validated since the DYSF label did not associate with the Golgi label. However, this experiment showed that the Golgi compartment in fused BeWo cells was most unusual. In IFM preparations, the Golgi in fused cells appeared to be expanded when compared to control non-fused cells. We used immunoblot assays to determine if marker-proteins for the Golgi complex were increased following cell fusion finding them increased. A Golgi function is the post-translational modification of newly synthesized proteins. This being the case we next asked if the endoplasmic reticulum (ER) was also expanded. Indeed both IFM and immunoblot assays showed ER marker proteins increased expression. It appears that both of these organelles,

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the Golgi complex and ER expand in parallel. A logical extension of this line of inquiry was to ask if a specific protein known to be processed by the Golgi also displayed enhanced expression. Human chorionic gonadotropin (hCG) production is a marker of trophoblast differentiation. In immunoblot assays, cell-associated hCG was not detectable in control BeWo cells. However, there was a massive increase in hCG expression by 24 hours forskolin treatment. With IFM we found high levels of hCG in both ER and Golgi complex.

It is critically important for cells to regulate the size and number of intracellular organelles. Several hypotheses have been put forward to account for this regulation. It has been proposed that organelle growth control is regulated through a limiting pool of cytoplasmic components[3,4] has suggested several possibilities to control organelle size, including limiting precursors. Additional possibilities raised were (a) constant growth which requires a power-law scaling factor, (b) feedback based on size measurement requiring a biochemical sensor to measure size, (c) feedback based on organelle function, and (d) self-balancing which is a function of the rates of assembly and disassembly. Senupta and Linstedt have focused on the control of Golgi size and conclude that the major factor setting size is abundance of secretory cargo[5]. While we do not know what sets the size of Golgi in fused BeWo cells, it is probable that cargo, like hCG, is an important factor.

References

1. Vandré DD, Ackerman WE 4th, Kniss DA, et al.: Dysferlin is expressed in human placenta but does not associate with caveolin. Biol Reprod 2007; 77: 533–542.
2. Robinson JM, Ackerman WE 4th, Vandré DD: While dysferlin and myoferlin are co-expressed in the human placenta, only dysferlin expression is responsive to trophoblast fusion in model systems. Biol Reprod 2009; 81: 33–39.
3. Goehering NW, Hyman AA: Organelle growth control through limiting pool of cytoplasmic components. Curr Biol 2012; 22: R330–R339.
4. Marshall WF: Organelle size control systems: From cell geometry to organelle-directed medicine. Bioessays 2012; 34: 721–724.
5. Senupta D, Linstedt AD: Control of organelle size: The Golgi complex. Ann Rev Cell Develop Biol 2011; 27: 57–77.

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