IJAE
Vol. 119, n. 1 (Supplement): 75, 2014
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

An innovative in vitro co-culture of Caco2 and HT-29 cells for mimicking human intestinal epithelium: a morphological analysis

Elena Donetti, Francesca Arnaboldi, Laura Cornaghi, Paola De Luca, Margherita Maggioni, Amelia Fiorilli, Anita Ferraretto

Department of Biomedical Sciences for Health, Università degli Studi di Milano, 20133 Milan, Italy

Intestinal epithelium represents the physiological interface between our body and nutrients with their associated molecules. The necessity to evaluate this interaction together with the difficulties encountered using in vitro primary cultures of bioptic intestinal fragments led to the development of cell lines obtained by colon adenocarcinoma and established in culture. Among these, HT-29 e Caco2 cell lines are the most used. We previously demonstrated that i) subcultured Caco2 cells form a regular monolayer with microvilli and a well-developed junctional apparatus, indicating their absorbent properties (Ferraretto et al., 2007) and ii) differentiated HT-29 cells are a heterogeneous population in which both muco-secreting, entero-endocrine, and absorptive cytotypes co-exist (Gravaghi et al., 2007). Considering that a single cell line can not represent a complete model, we decided to co-culture the two cell lines in RPMI medium and at post confluency in order to have differentiated cells mimicking as near as possible the morpho-physiological intestinal microenvironment. After plating, cells were harvested at 0, 3, 6, 10, and 14 days of post confluence (T0, T3, T6, T10, and T14, respectively) for ultrastructural analysis by transmission electron microscopy (TEM). TEM observations strongly suggest that co-cultures display original features from T0 to T14, with cells initially poorly differentiated but which progressively formed multilayers (from T6) and present: i) microvilli (from T6); ii) a complete junctional apparatus (from T6), in particular desmosomes, (T14); iii) mucus granules (from T3). In parallel, immunofluorescence analysis for the expression of occludin, E-cadherin, and desmocollin 2 is ongoing to evaluate the molecular composition of cell junctions. Our results clearly indicates that co-cultures of HT-29 and Caco2 cells may represent a valid model of intestinal human epithelium characterized by the possibility to stop the cell growth at the post confluency time more appropriate depending on the application.

References

[1] Ferraretto et al. (2007) New methodological approach to induce a differentiation phenotype in Caco-2 cells prior to post-confluence stage. Anticancer Res 27:3919-3926.
[2] Gravaghi et al. (2007) Casein phosphopeptide promotion of calcium uptake in HT-29 cells – relationship between biological activity and supramolecular structure. FEBS Journal 274:4999-5011.

Keywords

In vitro model, transmission electron microscopy, cell junctions, immunofluorescence.