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Nutrient excess in a stabilized co-culture system of Caco2/HT-29 cells: an ultrastructural and functional time-course study

Anita Ferraretto, Michela Bottani, Laura Cornaghi and Elena Donetti

Department of Biomedical Sciences for Health, University of Milan, Italy

The intestine represents one of the most important barriers of our body and interacts with several exogenous substances, among which nutrients. Today, the effects due to an excess of nutrients on intestinal morpho-functional changes, similar to the ones found in obesity, have been studied only in *in vivo* animal models. Many experimental difficulties hampered in establishing a physiological long-term experimental model starting from primary cultures of normal small intestinal and colon cells. For this reason, an intestinal Caco2/HT-29 (70/30) co-culture was set up in our lab starting from the differentiated parental cell populations to mimic the human intestinal epithelium. Co-culture was harvested at confluence (T0) and at 3, 7, and 15 days (T3, T7, and T15, respectively) post-confluence. Ultrastructural (TEM) and functional analysis (Alkaline Phosphatase, ALP; Aminopeptidase N, APN; Dipeptidyl Peptidase-IV , DPPIV; Transepithelial Electrical Resistance, TEER) were carried out. In the present study, two parallel experimental groups were cultured: the standard group and the excess group. In the standard group, the culture medium was changed every four days, whilst in the excess group on alternate days from T0. Transmission electron microscopy revealed that the excess of nutrients drives co-cultures towards a less differentiated absorptive phenotype. On the other hand, mucus granule presence was more and more evident from T3. The specific activity of ALP and APN, known markers of intestinal differentiation, and that of DPPIV, a specific marker of enterocyte differentiation, progressively increased. TEER, indicative of the barrier properties of the co-culture, increased at post confluence up to T15. In conclusion, data here presented show that the excess of nutrients can directly modify both morphology and function of the intestinal cells, opening the way to study at the effects due to specific nutrients on cell proliferation and differentiation involved in the acquisition of an obese human phenotype.

Keywords

Transmission electron microscopy, cell differentiation, hydrolase activity