上皮生長因子對人類結腸腺癌(Caco-2)細胞總 RNA、總蛋白質含量及白胺酸胺基肽酶的影響

Effects of Epidermal Growth Factor on Total Cellular RNA, Protein and Leucine Aminopeptidase in Human Colon Adenocarcinoma (Caco-2) Cells

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摘要

體內與體外實驗顯示，人奶中之上皮生長因子(epidermal growth factor, EGF)對胎兒腸細胞的生長與發育具調節之作用。本研究目的為探討 EGF 對腸細胞生長及蛋白質消化酵素的影響，以人類結腸腺癌細胞(Caco-2)為實驗模型，分別添加 0、5（相當於人奶 EGF 濃度）、50（相當於初奶 EGF 濃度）或 250nM EGF 後，分析細胞總 RNA 與蛋白質含量，及白胺酸胺基肽酶(leucine aminopeptidase; L-leucyl-peptide hydrolase; EC 3.4.l.1)活性與含量。結果顯示：添加 EGF 24 小時後，細胞總 RNA 量隨著 EGF 濃度增加而上升為控制組的 1.6 至 3.1 倍，而 250 nM EGF 組的細胞總 RNA 量明顯比控制組和 5 nM EGF 組為高。添加 5 nM EGF 組的細胞總蛋白質含量則較控制組與添加 250 nM EGF 組為高。添加 EGF 組的白胺酸胺基肽酶比活性稍微降低為控制組的 78% 至 86%。而以 10%十二基硫酸鈉電泳法(SDS-PAGE)分離白胺酸胺基肽酶(53 kDa)，並用西方墨點法(Western blotting)分析結果亦顯示：添加 EGF 組的白胺酸胺基肽酶含量降低為控制組的 86% 到 93%。總結之，250nM EGF 可以明顯增加細胞總 RNA 含量，而添加 5nM EGF 可增加細胞總蛋白質含量，但添加 EGF(5-250 nM)對細胞膜上白胺酸胺基肽酶之比活性與含量並無明顯的影響。

Abstract

Both in vivo and in vitro studies have showed that epidermal growth factor (EGF) in human milk could modulate growth and development of fetal intestinal cells. The purpose of this study was to investigate the effect of EGF on growth and the protein digestion enzyme -leucine aminopeptidase (LAP) in human colon adenocarcinoma (Caco-2) cells. The total cellular RNA and protein, and the activity and content of LAP were examined after the addition of EGF at 0, 5 (close to EGF concentration in human milk), 50 (close to EGF concentration in colostrum) or 250 nM. After incubation with EGF 24 h, the total cellular RNA in the EGF treatment groups was elevated to 1.6- to 3.1-fold of the control group as the concentrations of EGF were increased. The 250 nM EGF treatment group had significantly higher total cellular RNA as compared to the control and 5 nM EGF treatment groups. The 5 nM EGF treatment group had higher total cellular protein than the control and 250 nM EGF

treatment groups. The LAP specific activity in the EGF treatment groups was 78~86% of the control group. In addition, LAP (53 kDa) content in the EGF treatment groups was 86-93 o of the control group after separation by 10% SDS-PAGE and analysis by Western blotting. In conclusion, 250 nM EGF treatment increased total cellular RNA and 5 nM EGF treatment increased total cellular protein; however, EGF treatments (5 to 250 nM) had no remarkable effects on the specific activity and content of LAP on the cell membranes of Caco-2 cells.