p38 Mitogen-Activated Protein Kinase 在過度醣化最終產物誘導

RAW 264.7 巨噬細胞表現環氧酵素-2 所扮演的角色

Involvement of p38 Mitogen-Activated Protein Kinase in Advanced Glycosylation End Products-Induced Cyclooxygenase-2 Expression in RAW 264.7 Macrophages

中文摘要

過度醣化最終產物(advanced glycosylation end products, AGEs)被認為與老化及長期糖尿病病人體內所形成之蛋白質結構改變、功能異常有關。在本篇論文中，我們將大白鼠 RAW 264.7 巨噬細胞給予 poly-L-lysine (PLL)-AGEs 處理以探討環氧酵素-2 (cyclooxygenase-2, COX-2)蛋白的表現。在外加花生四烯酸(arachidonic acid)的情況下測量前列腺素 E2 (prostaglandin E2, PGE2)的產量可發現 PLL-AGEs 在 RAW 264.7 巨噬細胞裡可促使環氧酵素-2 活性呈劑量、時間依賴性的增加。PLL-AGEs 亦可引發環氧酵素-2 蛋白的表現，然而此作用並不影響環氧酵素-1 (cyclooxygenase-1, COX-1)蛋白的表現。Gamma-glutamylcysteine synthetase 的抑制劑—L-buthionine-[S, R]-sulfoximine (BSO)或 glutathione 的前驅物—L-nitro-acetyl-cysteine (L-NAC)並不會影響 PLL-AGEs 刺激環氧酵素-2 蛋白的表現，表示 PLL-AGEs 所引發之環氧酵素-2 蛋白的表現並不源自於氧化壓力。另外，環氧酵素-2 蛋白的表現也不受一氧化氮合成酶(nitric oxide synthase, NOS)之競爭型抑制劑—L-gamma-nitro-L-arginine methyl ester (L-NAME)以及脂多醣體(lipopolysacchride, LPS)之抑制劑—polymyxin B 所抑制，表示此作用也不是經由誘導型一氧化氮合成酶的誘導或脂多醣體的污染而來。Tyrosine kinase 的抑制劑—genistein 和 tyrphostin AG 126 以及 p38 mitogen-activated protein kinase (MAPK)的抑制劑—SB 203580 可以抑制 PLL-AGEs 誘導環氧酵素-2 之蛋白表現，但 Ras 的抑制劑—FPT II 和 MEK 的抑制劑—PD 98059 對 PLL-AGEs 所誘導之環氧酵素-2 蛋白表現並沒有任何作用。利用 PLL-AGEs 刺激 RAW 264.7 巨噬細胞可活化 p38 MAPK，此反應可被 genistein 和 SB 203580 所抑制。綜合以上結果可知 protein tyrosine kinase 及 p38 MAPK 的活化的確參與 PLL-AGEs 誘導環氧酵素-2 蛋白表現之訊號傳遞路徑。

英文摘要

Advanced glycosylation end products (AGEs) have been implicated in the structural and functional alterations of proteins that occur during aging and long-term diabetes. In the present study, murine RAW 264.7 macrophages were incubated with poly-L-lysine (PLL)-AGEs to examine cyclooxygenase-2 (COX-2) protein expression. Treatment of RAW 264.7 cells with PLL-AGEs caused a dose-dependent increase in

COX activity as reflected by PGE2 secretion (measured in the presence of exogenous arachidonic acid). Furthermore, treatment of RAW 264.7 cells with PLL-AGEs induced COX-2 but not COX-1 expression. The induction was affected by neither L-buthionine-[S, R]-sulfoximine (BSO), a gamma-glutamylcysteine synthetase inhibitor, nor by L-nitro-acetyl-cysteine (L-NAC), a known glutathione precursor, suggesting that AGEs-induced COX-2 expression is not due to reactive oxygen species. Moreover, COX-2 expression was affected by neither N-gamma-nitro-L-arginine methyl ester (L-NAME), a competitive inhibitor of nitric oxide synthase (NOS), nor polymyxin B, a lipopolysaccharide (LPS) inhibitor, suggesting that COX-2 induction is not secondary to iNOS induction or LPS contamination. The tyrosine kinase inhibitor, genistein and tyrphostin AG 126, and the p38 mitogen-activated protein kinase (MAPK) inhibitor, SB 203580, inhibited PLL-AGEs-induced COX-2 expression, while the Ras inhibitor, FPT inhibitor II, and the MEK inhibitor, PD 98059, had no effect on PLL-AGEs-induced COX-2 expression. Incubation of RAW 264.7 cells with PLL-AGEs resulted in activation of p38 MAPK, and this activation was suppressed by genistein and SB 203580. Taken together, our results suggest that activation of protein tyrosine kinase and p38 MAPK is involved in AGEs-induced COX-2 expression in RAW 264.7 macrophages.