Ras、Raf 和 ERK 路徑在 Thrombin 誘導肺部巨噬細胞一氧化氮合成?

表現的角色探討

The Role of Ras/Raf/ERK Pathway in Thrombin-Mediated Inducible Nitric Oxide Synthase Expression in Alveolar Macrophages

中文摘要

Thrombin 為一個多功能的絲胺酸蛋白?，可從受傷的血管釋放出來，為參與凝血反應重要的凝血因子。此外，thrombin 也可以?生多方面的細胞生理反應，其中包括調節發炎反應。根據先前研究指出，在肺部巨噬細胞中，thrombin 可誘導一氧化氮合成? (inducible nitric oxide synthease, iNOS) 的表現，然而其中的作用機制仍不清楚。本論文主旨在探討 thrombin 刺激肺部巨噬細胞產生一氧化氮合成?表現是否透過 Ras/Raf/ERK 及 I B kinase (IKK)/nuclear factor- B (NF- B) 路徑而來。實驗結果證實，thrombin 會隨時間及濃度增加而誘導 NR8383 肺部巨噬細胞一氧化氮合成?的表現增加。Thrombin 誘導一氧化氮合成?的表現會被 Ras 抑制劑 (Manumycin A)、 功能性突變之 Ras (Ras N17)、 Raf 抑制劑 (GW5074)，及 MEK 抑制劑 (PD98059) 所抑制。Thrombin 所誘導 Ras 活性的增加會被 manumycin A 所抑制。Thrombin 誘導 Raf-1 在 Serine 338 位置磷酸化的現象也會被 manumycin A 及 GW5074 所抑制，相同地，thrombin 所誘導 ERK 磷酸化也會被 manumycin A、GW5074 及 PD98059 所抑制。接著進一步證實，NF- B 抑制劑 (PDTC)、I B phosphorylation 抑制劑 (Bay117082)及過度表現 I BM (I B mutant)皆可抑制 thrombin 所誘導的一氧化氮合成?的表現。NR8383 細胞經由 thrombin 刺激會促使 IKK / 磷酸化、I B 磷酸化、I B 降解以及 B-luciferase 活性增加。Thrombin 所誘導 IKK / 磷酸化及 B-luciferase 的活化同樣也會被 manumycin A、GW5074 及 PD98059 所抑制。綜合以上的實驗結果，顯示在 NR8383 肺部巨噬細胞中，thrombin 可經由 Ras/Raf/ERK 及 IKK / /NF- B 之訊息傳遞路徑來媒介誘導性一氧化氮合成?的表現。

英文摘要

Thrombin, a multifunctional serine protease generated at sites of vascular injury, and known for its pivotal role in the coagulation cascade, contributes to tissue repair, but also promotes a wide range of cellular responses including modulation of the inflammatory responses. Previous reports showed that thrombin induced inducible nitric oxide synthase (iNOS) expression in lung macrophages; however, the signal pathway is still unclear. This study investigated the Ras/Raf/ERK and I B kinase (IKK)/nuclear factor- B (NF- B) signaling pathways involved in iNOS expression by thrombin in NR8383 alveolar macrophage. Thrombin caused increase in iNOS expression in a time- and concentration- dependent manner. Thrombin-induced iNOS expression was inhibited by Manumycin A (Ras inhibitor), dominant negative matant of Ras (Ras N17), GW5074 (Raf inhibitor), and PD98059 (MEK inhibitor). The thrombin-induced increase in Ras activity was inhibited by manumycin A. Raf-1 phosphorylation at serine 338 residue by thrombin was inhibited by manumycin A and GW5074. The thrombin-induced ERK phosphorylation was also inhibited by manumycin A, GW5074, and PD98059. Furthermore, pretreatment of PDTC (NF- B inhibitor) or Bay117082 (I B phosphorylation inhibitor) or overexpression of I BM (I B mutant) all inhibited thrombin-induced iNOS expression. Stimulation of NR8383 cells with thrombin induces increase in IKK / phosphorylation, I B phosphorylation, I B degradation, and B-luciferase activity. The thrombin-induced increase in IKK / phosphorylation and B-luciferase activity was inhibited by manumycin A, GW5074, and PD98059. These results indicated the Ras/Raf/ERK and IKK / /NF- B signaling pathways involved in thrombin-induced iNOS expression in NR8383 cell.