嗜中性彈性蛋白酶藉由誘發 NF-κB 抑制因子降低人類呼吸道平滑肌細胞介白素 8 的合成

Neutrophil Elastase Represses IL-8/CXCL8 Synthesis in Human Airway Smooth Muscle Cells through Induction of NF-κB repressing factor (NRF)

中文摘要

嗜中性球 (neutrophil) 之浸潤是許多急慢性呼吸道發炎的重要之病理變化，活化後的 neutrophil 會釋放許多發炎介質，包括嗜中性彈性蛋白酶 (neutrophil elastase)。呼吸道平滑肌細胞 (human airway smooth muscle, hASM) 收縮會造成呼吸道狹窄，是導致氣喘病患呼吸道過敏以及臨床症狀的最關鍵因素。過去的研究認爲呼吸道平滑肌細胞的作用有引發支氣管收縮、分泌細胞激素、趨化激素和表達細胞黏著因子，用以調節黏膜下呼吸道發炎反應，呼吸道平滑肌細胞同時也是很多發炎介質的來源，調節呼吸道的發炎和進展。過去我們的研究發現，neutrophil elastase 在人類呼吸道平滑肌細胞會活化 NF-κB 進而誘發 TGF-β1 的表現。同屬於 NF-κB 媒介的 IL-8/CXCL8 是否也會受 neutrophil elastase 刺激而增加分泌?

本研究進一步探討在人類呼吸道平滑肌細胞被活化的 NF-κB 對 IL-8/CXCL8 的基因反應，發現 IL-8/CXCL8 的釋放和 mRNA 的表現都被 neutrophil elastase 抑制，若先加入轉錄抑制劑 actinomycin D 後再以 neutrophil elastase 刺激，結果得到相似趨勢的 IL-8/CXCL8 mRNA 分解速率，證明 neutrophil elastase 並未影響 IL-8/CXCL8 mRNA 之穩定度，因而推論這屬於轉錄層次的作用。本研究進而探討其中之分子機制。 NF-κB 抑制因子 NRF (NF-κB repressing factor) 爲抑制 NF-κB 之 DNA 結核蛋白。目前對於 NRF 相關調節機轉及角色了解有限，已經被證實他抑制許多基礎狀態下 NF-κB 所影響的特定基因，包括 IL-8/CXCL8、 iNOS 和 interferon-β 本研究假設 neutrophil elastase 透過 NRF 而抑制 IL-8/CXCL8 基因之表現。反轉錄-聚合酶連鎖反應 (RT-PCR) 和西方墨點 (WB) 顯示 neutrophil elastase 會引發 NRF 的表現，運用染色質免疫沈澱法 (chromatin immunoprecipitation or ChIP) 並證實 NRF 接合到 IL-8/CXCL8 啓動子 (promoter) 上，然後再使 RNA Pol 2 從 IL-8/CXCL8 promoter 上移除。進一步並發現 NRF siRNA 可以阻斷 neutrophil elastase 對 IL-8/CXCL8 的抑制作用。相反的，在呼吸道上皮細胞 A549 和 Beas-2B 以 neutrophil elastase 刺激卻增加 IL-8/CXCL8 的表現。在這些細胞中 NF-κB p65 被活化卻無法誘發 NRF 的表現。以人爲的方式將 NRF (pNRF) 質體轉殖到呼吸道上皮細胞 A549 使其 NRF overexpression 證實 NRF 可抑制 IL-8/CXCL8 的表現。

研究接著發現將 NF-κB 的上游訊息傳導路徑阻斷或用 NF-κB 的細胞核轉移

抑制劑 SN50 可反轉 neutrophil elastase 對 IL-8/CXCL8 的抑制，顯見 neutrophil elastase 抑制 IL-8/CXCL8 的作用乃透過活化 NF-κB 。活化有時像似 Janus-faced 一樣，因不同的變異次單位的介入而出現截然不同的結果。我們研究發現嗜中性球分泌出來的 neutrophil elastase，在人類呼吸道平滑肌細胞抑制 IL-8/CXCL8 的表現，主要是間接透過 RelB-associated dimers 的反應。然而 neutrophil elastase 若刺激肺部上皮細胞 A549 使 IL-8/CXCL8 分泌增加，主要是透過 p65-associated dimmers 的結果。相對的，利用 IL-1β刺激呼吸道平滑肌細胞，也可以藉由活化 p65-associated dimmers 而增加 IL-8/CXCL8 的表現。本研究進一步假設 neutrophil elastase 刺激呼吸道平滑肌細胞抑制 IL-8/CXCL8 的表現， 乃透過 RelB 誘發 NRF，再藉由 NRF 結合在 IL-8/CXCL8 promoter 上，促使 RNA pol 2 從 IL-8/CXCL8 promoter 上被移除。此假說在 siRelB 可減弱 neutrophil elastase 之表現及 IL-8/CXCL8 的抑制作用獲得證實。利用 ChIP 並發現 neutrophil elastase 可引起 RelB 結合到 NRF 之啓動子並伴隨著 RNA pol 2 結合量之增加，證實 RelB 爲引發 NRF 基因轉錄之轉錄因子。

綜合以上發現，本研究提出一個新的負向調節機轉—在人類呼吸道平滑肌細胞，neutrophil elastase 藉由 RelB-associated dimmers 引發 NRF 而抑制 IL-8/CXCL8 表現。此研究不僅讓吾人對人體呼吸道發炎的內在控制機轉有更新更深一層的認識，並提供一個可運用來治療疾病的新標的。

英文摘要

Neutrophils are recruited to the airways and play a role in the pathogenesis of chronic airway diseases, such as COPD, cystic fibrosis and more severe form of asthma. Elastase released by neutrophils is implicated in such chronic conditions. ASM has long been regarded as having mainly contractile properties in response to many proinflammatory mediators and neurotransmitters. Studies now show that ASM is also a source of inflammatory mediators through its synthetic functions, through which may regulate the development and progression of airway inflammation. NF-κB repressing factor (NRF), a nuclear inhibitor of NF-κB, is constitutively expressed and is implicated in the basal silencing of specific NF-κB targeting genes, including IFN-β, IL-8/CXCL8, and iNOS. Little is known about the regulation of itself and its role in response to stimuli. We have previously reported that neutrophil elastase (NE) activates NF-κB in primary human ASM (hASM), leading to induction of TGF-β1. It is describe here that, instead of inducing the NF-κB response gene IL-8/CXCL8, NE suppressed IL-8/CXCL8 release and mRNA expression in hASM cells. Transcriptional blockade studies using Actinomycin D revealed a similar degradation rate of IL-8/CXCL8 mRNA in the presence or absence of NE, suggesting an involvement at the transcription level. Mechanistically, the NE repressive effect was mediated by inducing NRF, as shown by RT-PCR and Western blotting, which was

subsequently recruited to the native IL-8/CXCL8 promoter leading to removal of RNA polymerase II (RNA Pol 2) from the promoter, as demonstrated by chromatin-immunoprecipitation (ChIP) assays. Knockdown of NRF by siRNA prevented NE-induced suppression of IL-8/CXCL8 expression. In contrast, NE did not induce NRF expression in A549 and Beas-2B cells, where NE only stimulates NF-κB activation and IL-8/CXCL8 induction. Forced expression of NRF in A549 cells by a NRF expression plasmid suppressed IL-8/CXCL8 expression.

The consequence of NF-κB activation is cell and context dependent and sometimes Janus-like. This is in part due to involvement of variable subunits which have distinct and non-overlapping functions. It is therefore possible that activation of different NF-κB subunits confer the differential effect of NE between ASM and airway epithelial cells. Here, it is demonstrated that NE specifically inhibits the expression of the proinflammatory chemokine IL-8/CXCL8 in primary hASM cells indirectly by utilising RelB-associated dimers. In contrast, NE stimulates IL-8/CXCL8 expression in lung epithelial cells through activation of p65-associated dimers. A similar effect is also seen in IL-1β-stimulated primary hASM cells. Mechanistically, the RelB repressive is mediated by inducing NRF which is subsequently recruited to the native IL-8/CXCL8 promoter leading to removal of RNA pol 2 from the promoter. Knockdown of NRF by siRNA prevents NE-induced suppression of IL-8/CXCL8 expression. NE-activated RelB is recruited to the native NRF promoter and knockdown of RelB by siRNA attenuates NRF induction by NE. Hence we describe a novel negative regulatory mechanism of RelB-induced NRF which has a potential anti-inflammatory effect.

In conclusion, this report has described a novel negative regulatory mechanism of NE-induced NRF which is restricted to hASM and mediates RelB-activated NRF up-regulation the suppression of IL-8/CXCL8 expression.