特定蛋白激酶;C 亞型的活化決定人類周邊血液單核球細胞的命運：

使之趨向特定分化途徑或進行計畫性細胞凋亡

The Activation of Specific Protein Kinase C Isoform(s) Determining the Cell Fate of Human Peripheral Blood Monocytes towards Differentiation or Apoptosis

中文摘要

人類周邊血液之單核球細胞受到適當的刺激時，會分化成爲單核球細胞衍生之巨嗜細胞（Monocyte-derived macrophages，簡稱 MDMs）或樹突細胞（Monocyte-derived dendritic cells，簡稱 MoDCs）。然而，單核球細胞分化成爲 MDMs 及 MoDCs 的過程中，哪些細胞內的分子扮演關鍵角色，目前仍不清楚。因此，本論文研究方向之一就是要釐清蛋白質激酶 C 亞型群（Protein kinase C isoenzymes，簡稱 PKCs）在單核球細胞分化成爲 MDMs 及 MoDCs 過程中所扮演的角色。在本研究中，我們發現以 GM-CSF（103 IU/ml）或 PMA（10 nM）處理人類周邊血液單核球細胞時，會觸動細胞內 PKCα之活化及轉位（translocation），並且誘發單核球細胞分化成爲 MDMs。進而發現當以 PKCα/β抑制劑-Go6976 來處理單核球細胞後再加入 GM-CSF 時，GM-CSF 所引發之細胞內 PKCα轉位及單核球細胞分化爲 MDMs 明顯地受到抑制。另一方面，分別以 Go6976 或 PKCβ專一性抑制劑來對單核球細胞做前處理後，再加入 GM-CSF 及 IL-4 時，發現兩種抑制劑皆明顯地抑制了 GM-CSF 加 IL-4 所誘導之單核球細胞分化爲 MoDCs 的現象。進一步的實驗顯示，以 GM-CSF 及 IL-4 處理單核球細胞時，會促使細胞內 PKCβ1 之活化及轉位。因此，根據以上實驗結果，得知人類周邊血液單核球細胞內 PKCα或 PKCβ1 的活化可誘導該細胞分別趨向分化爲 MDMs 或 MoDCs。

另一方面，以高濃度（>100 nM）之 PMA 處理細胞時，會誘導許多不同細胞趨向計畫性細胞凋亡（Apoptosis）之機制。因此，本論文之另一研究方向是要釐清不同濃度之 PMA 對人類周邊血液單核球細胞內之 PKC 亞型群及單核球細胞功能的影響。結果顯示低濃度（1-10 nM）之 PMA 會觸動細胞內 PKCα之活化，並且誘發單核球細胞分化成爲 MDMs。但是，以 1,000 nM 之 PMA 濃度處理單核球細胞時，會活化細胞內 PKCβ2，並且促進單核球細胞趨向 Apoptosis 之機制。

以上實驗結果證實當特定 PKCα或 PKCβ1 被活化時，可分別誘導人類周邊血液單核球細胞趨向分化爲 MDMs 或 MoDCs。然而，當細胞內 PKCβ2 被活化時，則會引導單核球細胞走向 Apoptosis 之路徑。

英文摘要

Human peripheral CD14+-monocytes have been known to differentiate into monocyte-derived macrophages (MDMs) or dendritic cells (MoDCs) upon suitable stimulation. However, the key intracellular molecule(s) associated with their differentiation fates was (were) not fully understood. This study was designated to

determine the association of PKC isoenzymes with the differentiation of CD14+-monocytes into MDMs or MoDCs. The treatment of CD14+-monocytes with either granulocyte/macrophage colony-stimulating factor (GM-CSF) (103 IU/ml) or phorbol-12-myristate-13-acetate (PMA) (10 nM) elicited PKCα translocation and consequently induced their differentiation into MDMs. The inclusion of PKCα/β specific inhibitor, Go6976, greatly inhibited the GM-CSF-induced PKCα translocation and dose-dependently reduced the GM-CSF-induced MDM differentiation. Furthermore, the simultaneous pretreatment of CD14+-monocytes with Go6976 and PKCβ-specific inhibitor predominantly suppressed the GM-CSF/interleukin-4 (IL-4)-induced generation of MoDCs. Further study demonstrated that GM-CSF/IL-4 selectively induced the translocation of PKCβ1, not PKCα or PKCβ2, in CD14+-monocytes. These results indicate that the cell fate commitment of CD14+-monocytes towards MDMs or MoDCs appears to be steered by the selective activation of PKCα or PKCβ1, respectively.

On the other hand, it has been known that PMA at high concentration (< 100 nM) induces cell apoptosis in many cell types. Therefore, it was attempted to delineate the possible role of PKC isoforms in PMA-induced changes in cellular fate of CD14+-monocytes. Our results showed that PMA at 1,000 nM predominantly induced the apoptosis of CD14+-monocytes. The further studies identified that the activation of PKCβ2 was essential for the PMA-induced apoptosis of CD14+-monocytes.

In conclusion, our data demonstrate that the selective activation of PKCα results in the differentiation of CD14+-monocytes into MDMs. The specific activation of PKCβ1 leads CD14+-monocyte differentiation toward MoDCs. Moreover, the activation PKCβ2-dependent signaling induces the programmed cell death of CD14+-monocytes.