行政院國家科學委員會專題研究計畫　成果報告

Toll-like接受體-9及其受器在多發性骨髓瘤的生物效應及可能的臨床應用

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一、基本資料 **計畫編號：NSC93--2314-B-002-087**

計畫種類	一般型研究計畫，A類(研究主持費及執行計畫所需經費)			
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計畫名稱 中文	Toll-like 接受體-9 及其受器在多發性骨髓瘤的生物效應及可能的臨床應用			
計畫名稱 英文	Biological and clinical implication of toll-like receptor 9 (TLR-9) and its ligand in multiple myeloma			
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本研究計畫發表情形:	□ 論文已發表	□ 投稿中	□ 學會發表	■ 未發表

計畫成果摘要:

計畫成果(限四頁以內)

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I. 簡介（Introduction）與背景說明

Multiple myeloma (MM) is a clonal plasma cell neoplasm characterized by proliferation of abnormal plasma cells in bone marrow (BM) that secrete a monoclonal paraprotein (M-protein) in serum and/or urine, and by osteolytic bone destructions. So far, it is an incurable disease and its pathogenesis is largely unknown. The median survival time of MM patients is only three to four years, and then the patients will die on either the disease or its complications. Bacterial infection, usually occurred in the late stage of MM, is the leading reason for mortality. There have been several independent prognostic factors for MM, and one of it is the extent of angiogenesis in BM [1]. Myeloma cells have been reported to be with pro-angiogenic activity [2], but the involved molecules and regulatory mechanisms were still unclear.

The **mammalian toll-like receptors (TLRs)**, originally described for their homology with the Drosophila toll [3], are a family of transmembrane receptors containing extracellular leucine-rich regions, which recognize various microbial components engaging a signaling cascade that results in the response versus such microbes. Exposure to microbial products and cytokines regulate TLRs expression with considerable species-related differences [4]. In human and other mammalians, of the 10 TLRs described to date, different ligands of 9 of the 10 TLRs were identified. Members of the TLR family are differentially expressed on hematopoietic and nonhematopoietic cells [5]. In general, mononuclear phagocytes and dendritic

cells express the widest TLR repertoire [5]. TLRs respond to bacteria and bacterial products by transmitting a ligand induced trans-membrane signal that induces the expression of cytokines such as TNFα, IL-1, IL-6, and IL-12 that are important in the host response to infection [6]. Unmethylated CpG motifs (non-methylated C-G dinucleotides flanked by two 5' purines and three 3' pyrimidines), characteristic of bacterial DNA, are detected by **TLR9**. Many recent studies have focused on CpG motif and its role as stimulators of immune cells [7] and several studies have suggested a role for TLRs in the stimulatory effects induced by microbial products on human B lymphocytes [8], however, the expression of TLRs in B cells has not been systematically investigated until very recently, a distinct TLR mRNA expression profile, which includes particularly high levels of **TLR9** and TLR10, was found on normal and malignant B cells, including MM [9]. Engagement of the B cell receptor or the costimulatory molecule CD40, augmented the **TLR9** transcript in resting B cells. Augmented TLR9 mRNA expression was associated with increased responsiveness to its agonist, CpG motif, enhancing proliferation and chemokine production of the immune cells. The regulated expression of selected TLRs in B cells may play an important role in linking innate to adaptive immune responses [8,9]. Therefore, the TLR family of receptors thus appears to provide a critical interface between higher organisms and microorganisms, whereby microorganisms, or distinct components thereof, are recognized, signal cellular activation, and induce a host response against these organisms.

TLR-dependent signaling pathways lead to the activation of the IκB/NF-κB pathway, inducing the expression of genes that participate in innate immune responses, including many inflammatory cytokines and antimicrobial peptides [10]. Very interestingly, an angiogenic switch in macrophages involving synergy between **TLR9** and adenosine A_{2A} receptors ($A_{2A}R$) were found [6], which hinted that TLR9 might play some role in angiogenesis with coexistence of other molecules. In the absence of adenosine or $A_{2A}R$ agonists, unmethylated CpG motif (**TLR9** agonist) strongly up-regulate TNFα expression, with no effect on vascular endothelial growth factor (VEGF). On the contrary, in the presence of adenosine or $A_{2A}R$ agonists, **TLR9** agonist strongly up-regulate VEGF expression, while simultaneously down-regulating TNFα [6]. For **TLR9** was possibly expressed by myeloma cells (MCs) [9], whether the angiogenic switch involving synergy between **TLR9** and $A_{2A}R$ also occurred in MCs and resulted in the pro-angiogenic activity of MCs is warranted further studies.

By a new xenotransplant animal model we have established for human multiple myeloma [11], we would like to study on the biological effects of **TLR9** and other co-receptors, like $A_{2A}R$, in MM.

II.關鍵材料及方法 (Subjects and Methods)

I. Immunostaining of TLR-9 on MM plasma cells

我們首先嘗試用免疫化學染色法來檢視是否病人的漿細胞和骨髓瘤細胞株的漿細胞上有無 TLR-9 的表現。由於 TLR-9 主要存在細胞質中，因此要做染色前必須在細胞表面上打洞，染色用的單株抗體才得以進入細胞質中。此種免疫化學染色法的作法可見我們先前的文獻發表[12]。我們收集到 10 例新診斷為 MM 的病案，取其骨髓檢體加以 Ficoll-Paque 溶液分離取得 mononuclear cells (MNCs) [11]一部分 MNCs 則利用磁珠吸附的原理(magnetic cell sorting)來純化

其中的 myeloma cells (MCs)。一部分純化的 MCs 作成短期細胞培養，並做染色 TLR-9 用。

II Real-time PCR of expression of TLR-9 gene from clinical samples of MM patients

另一部分之MCs則直接抽取RNA用real-time PCR的方式半定量其TLR-9的基因表現[9]。TLR9基因表現的狀況便會和病人臨床表現作比較，看臨床上是否有何特徵。

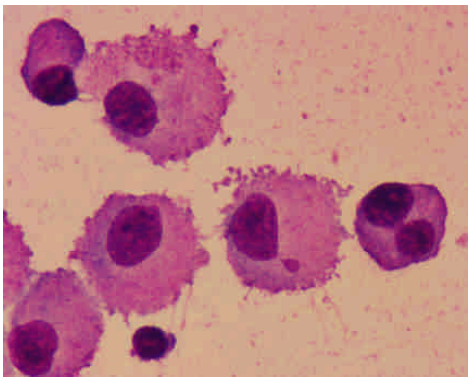
III.重要之結果 (Results)

1. 我們確定TLR-9幾乎表現在我們所有骨髓瘤病人和骨髓瘤細胞株的漿細胞細胞質中(除了細胞株RPMI-8226以外)(如圖一所示）。
2. real-time PCR的方式半定量這些檢體其TLR-9的基因表現則互有差異。
3. TLR-9的基因表現的多少與臨床上的特徵或是預後暫無關聯性。

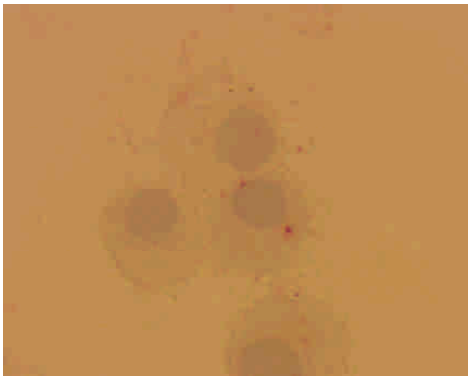
VI.討論 (Discussion)與成果之貢獻

1. 我們確定TLR-9可以表現在癌化的漿細胞上。
2. TLR-9的生物意義仍需更多的檢體和用其ligand刺激才能得知。

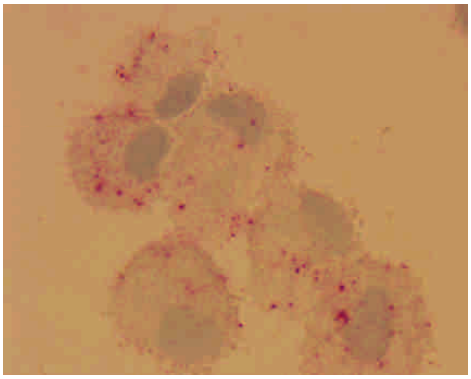
圖一



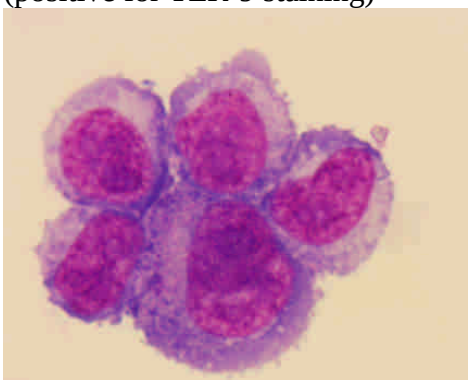
漿細胞型態，病人檢體，劉氏染色



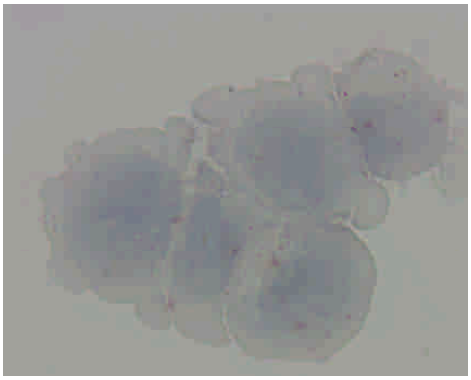
漿細胞，病人檢體，免疫化學染色 (negative control)



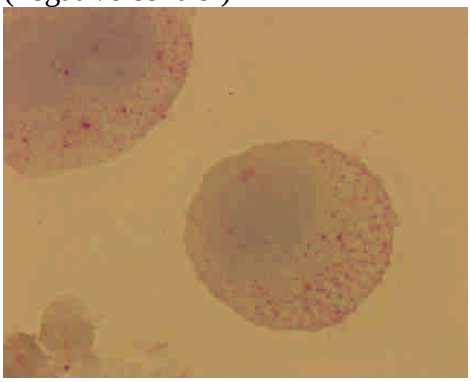
漿細胞，病人檢體，免疫化學染色
(positive for TLR-9 staining)



漿細胞型態，細胞株IM-9，劉氏染色



漿細胞，細胞株IM-9，免疫化學染色
(negative control)



漿細胞，細胞株IM-9，免疫化學染色
(positive for TLR-9 staining)

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