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幽門桿菌對乙型環氧酶;的活體外及活體內調控：機轉探察與胃癌致病機制的關係(2/3)
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行政院國家科學委員會專題研究計畫成果報告

幽門桿菌對乙型環氧酶的活體外及活體內調控：機轉探察與胃癌致病機制的關係(2/3)

In vitro and in vivo modulation of cyclooxygenase-2 by Helicobacter pylori: mechanistic insights and relationship to gastric carcinogenesis (2/3)

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中 文 摘 要

流行病學觀察顯示非固醇類抗發炎藥物的使用可以降低胃癌的危險性，而且幽門桿菌誘發乙型環氧酶的表現可能在胃癌致病機轉扮演重要角色。因此探討幽門桿菌感染、乙型環氧酶表現和胃癌之間的關係可能開創胃癌預防和治療的新契機。雖然如此，過去此方面的研究並不完整而且彼此之間相關的機轉也未清楚釐清。

吾人在過去研究已成功建立細胞株培養和動物模式，可供幽門桿菌誘發乙型環氧酶活化機轉的探討。利用這些已建立的模式，此三年計畫的目標在研究訊息傳導機制、尋找與乙型環氧酶攸關的蛋白，並且發展可以針對胃癌異常乙型環氧酶活性的預防與治療策略。於第一年的計畫中，吾人發現幽門桿菌誘發乙型環氧酶的表現可以經由 TLR2 和 TLR9 的作用加強癌細胞侵襲性和血管新生，而且此作用可被具專一性的乙型環氧酶抑制劑 NS398 和 celecoxib 減弱。在 MAPKs 調控乙型環氧酶的表現過程中，則是經由乙型環氧酶上的起動子 CRE 和 AP1，而非κB。利用 DNA affinity-protein binding, supershift 和 chromatin immunoprecipitation 等方法，吾人証實 CREB-1, ATF-2 和 c-jun 可與 CRE 作用，而 c-fos, c-jun 和 ATF-2 則和 AP-1 作用。這些轉譯因子的活化作用可以被不同 MAPKs 的抑制劑減弱。TLR2, TLR9 或 MAPKs 等的突變則可抑制幽門桿菌誘發乙型環氧酶的啟動子 CRE 和 AP-1 的活性。MAPKs 抑制劑可減弱幽門桿菌誘發乙型環氧酶的 mRNA 與蛋白表現。這些結果顯示幽門桿菌透過 TLR2 和 TLR9 以活化 MAPKs(尤其是 p38)和其下游的轉譯因子(CREB-1, ATF-2, c-jun 和 c-fos)，以達到活化位於乙型環氧酶啟動子上的 CRE 和 AP-1 的作用。上述的細胞內傳訊啟動乙型環氧酶有關的前列腺素 E2 釋放，而導致細胞的侵襲性和血管新生(圖 1)。

除了釐清幽門桿菌誘發的訊息傳導路徑和作用外，吾人另外也建立可過度表現乙型環氧酶的細胞株，並且利蛋白質體技術找到一些乙型環氧酶有關的蛋白(表 1)，我們以 RT-PCR 進一步分析 AGS，mock 和 COX-2 過度表現細胞株在上述基因的 mRNA 表現，結果顯示 NRG1 有過度表現，而 calreticulin、ERP29、HYOU1 和 angiopoietin-2 則有減少表現情形。(圖 2 及表 2)這些基因將進一步以 immunohistochemistry 在胃癌組織分析，若有變化，將做功能性、包括血管新生、腫瘤侵襲等分析。

關鍵詞：乙型環氧酶、幽門桿菌、血管新生、蛋白質體、胃癌致病機制

Abstract

Epidemiologic observations suggest that use of non-steroid anti-inflammatory drugs reduces the risk of gastric cancer. H. pylori-induced cyclooxygenase-2 (COX-2) expression plays a crucial role in gastric carcinogenesis. Investigations of relationship between H. pylori infection, COX-2 expression and gastric cancer might open up many new opportunities for cancer prevention and therapeutics. However, previous studies were incomprehensive and the linkage mechanisms between them have not been fully elucidated.

In the first grant period, we have found that H. pylori-induced COX-2 expression enhances the cancer cell invasion and angiogenesis via TLR2 and TLR9, which can be attenuated by the specific COX-2 inhibitor NS398 or celecoxib. The cAMP response element (CRE) and AP1 sites, but not κB on the COX-2 promoter, are involved in MAPKs-regulated COX-2 expression. Differential bindings of the CREB-1, ATF-2, c-jun to the CRE site, and the c-fos, c-jun, ATF-2 to the AP1 site are demonstrated. The mutants of TLR2, TLR9, or MAPKs inhibited H. pylori-induced COX-2 promoter, CRE, and AP-1 activities. MAPKs inhibitors attenuated the H. pylori-induced COX-2 mRNA and protein expressions. These results indicate that H. pylori acts through TLR2 and TLR9 to activate MAPKs, especially p38, and their downstream transcription factors (CREB-1, ATF-2, c-jun, and c-fos), resulting in the activations of CRE and AP-1 on the COX-2 promoter. These intracellular networks drive the COX-2-dependent PGE2 release and contribute to cell invasion and angiogenesis (Fig.1).

In addition to elucidation of the signaling pathways and actions of H. pylori-included COX-2 expression, we have established COX-2-overexpressing AGS cell line and utilized proteomic techniques to identify several COX-2 dependent proteins (Table 1). Using RT-PCR in AGS, Mock and COX-2 overexpressing cell line, we found overexpression of NRG1 gene and reduced expression of calreticulin, ERP29, HYOU1 and angiopoietin-2 (fig.2 & table 2) in COX-2 overexpressing cell lines. Immunohistochemical staining of these gene products in gastric cancer tissues will be performed. Those with altered expression will be selected for functional analyses including angiogenesis and tumor invasion assays.

Keywords: cyclooxygenase-2, H. pylori, angiogenesis, proteomic, gastric carcinogenesis

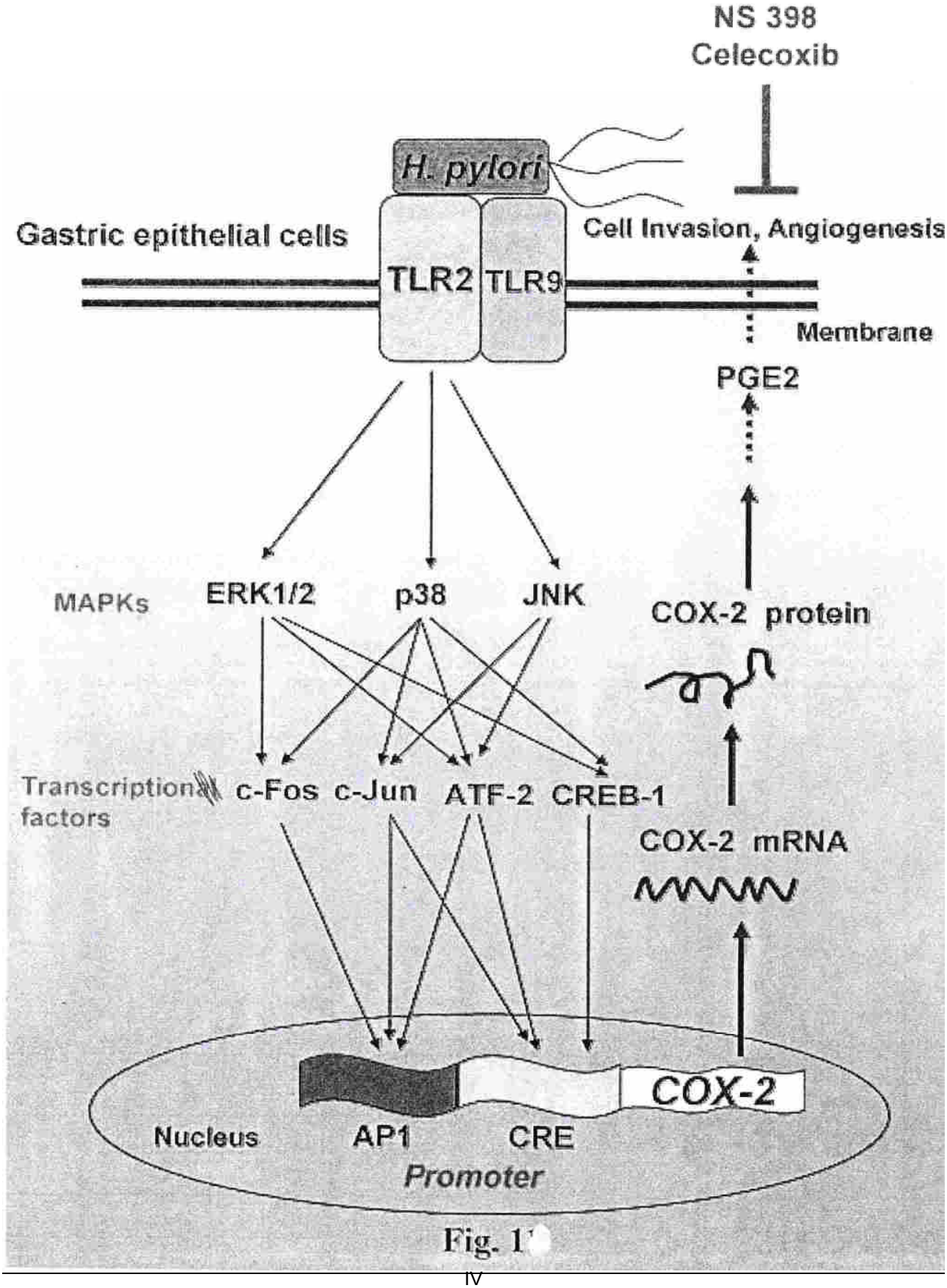
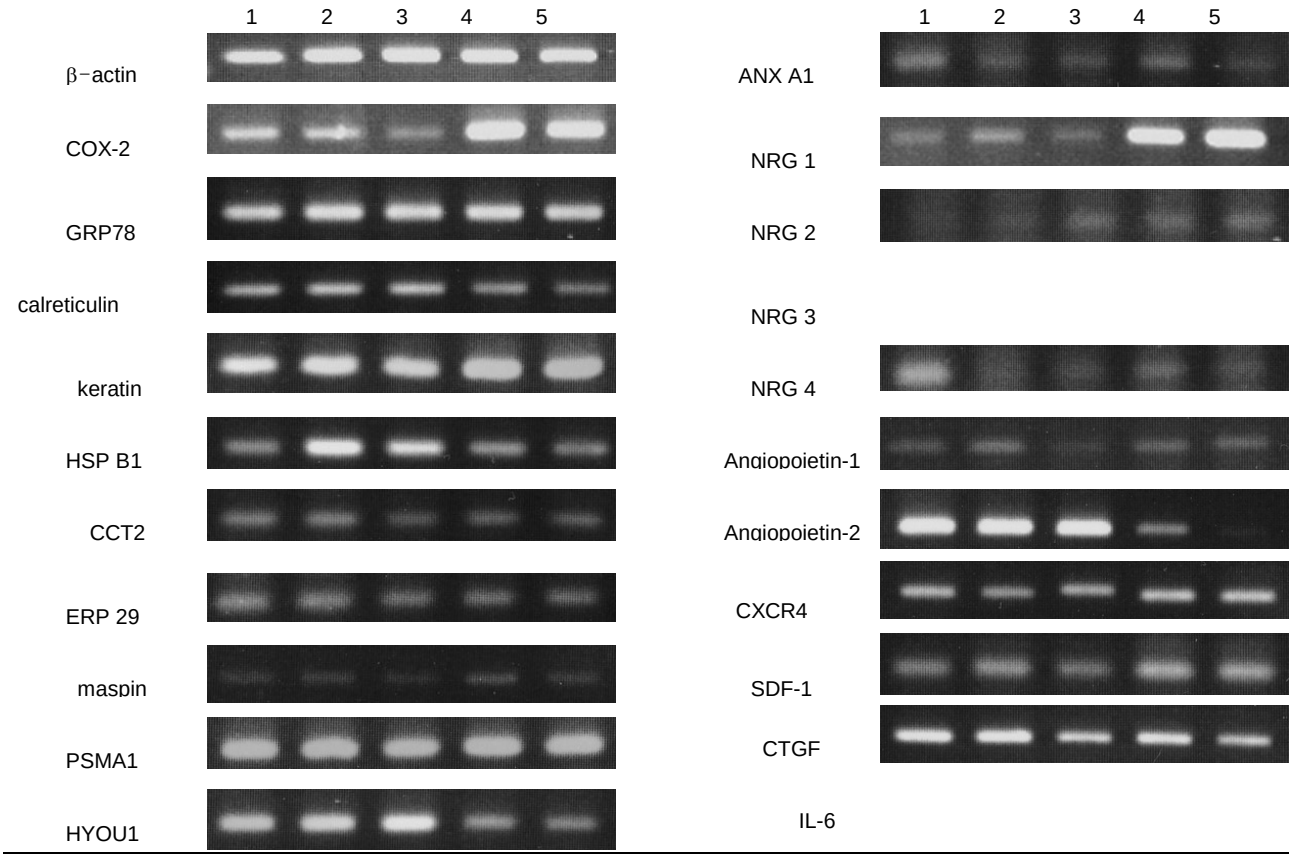

NS 398
Celecoxib
H. pylori
Gastric epithelial cells
TLR2
TLR9
Cell Invasion, Angiogenesis
Membrane
PGE2
MAPKs
ERK1/2
p38
JNK
COX-2 protein
Transcriptional factors
c-Fos
c-Jun
ATF-2
CREB-1
COX-2 mRNA
COX-2
Nucleus
AP1
CRE
Promoter

Fig. 1



Lane 1:WT AGS

Lane 2:mock 1-1

Lane 3:mock 1-2

Lane 4:COX-2 1-1

Lane 5:COX-2 1-2

Table 1. Potential proteins involved in COX-2 overexpression of gastric cancer identified by 2D

No	Protein
1.	78K Da glucose-regulated prtein precursor (GRP78)
2.	Calreticulin precursor (CRP55)
3.	Cytokeratin-18 (CK-18)
4.	Annexin A3
5.	Heat-shock protein beta-1 (HspB1)
6.	T-complex protein 1, beta subunit (TCP-1-beta)
7.	Endoplasmic reticulum protein Erp29 precursor (Erp31, Erp28)
8.	Maspin precursor (Protease inhibitor 5)
9.	Proteasome subunit alpha type 1 (Proteasome component C2)
10.	150kDa oxygen-regulated protein precursor (Orp 150, Hypoxia up-regulated 1)

Table 2.

cell lines	AGS	mock 1	mock 2	cox-2 1	cox-2 2
Lanes:	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5
actin	1	1.1	1.2	1	0.96
cox-2	1	1.2	0.58	1.9	1.7
GRP78	1	1.1	1.1	1	0.88
calreticulin	1	1.2	1.1	0.83	0.65
keratin	1	1.1	0.99	1.2	1.2
HSP B1	1	2.2	1.8	1	0.94
CCT2	1	1.1	0.65	0.67	0.66
ERP29	1	0.93	0.66	0.69	0.61
maspin	1	1.1	0.68	1.2	0.83
PSMA1	1	0.99	0.96	1.1	1
HYOU1	1	1.2	1.4	0.67	0.52
ANX A1	1	0.65	0.5	0.71	0.62
NRG1	1	2.7	1.6	4.1	4.7
NRG 2	1	1.2	1.8	2.1	2.2
NRG3					
NRG 4	1	0.39	0.34	0.51	0.48
angiopoietin-1	1	1.4	0.67	1.3	1.3
angiopoietin-2	1	0.96	0.89	0.27	0.055
CXCR4	1	0.72	0.83	1	1
SDF-1	1	1.3	0.9	1.7	1.4
CTGF	1	1.1	0.73	0.91	0.68
IL-6					

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