行政院國家科學委員會專題研究計畫 期中進度報告

D 型肝炎病毒 mRNA 體外轉錄系統製備：細胞 RNA 聚合酶之鑑定與病毒抗原的功能分析(2/3)
期中進度報告(精簡版)

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計 畫 編 號 ：NSC 95-2320-B-002-037-
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執 行 單 位 ：國立臺灣大學醫學院臨床醫學研究所

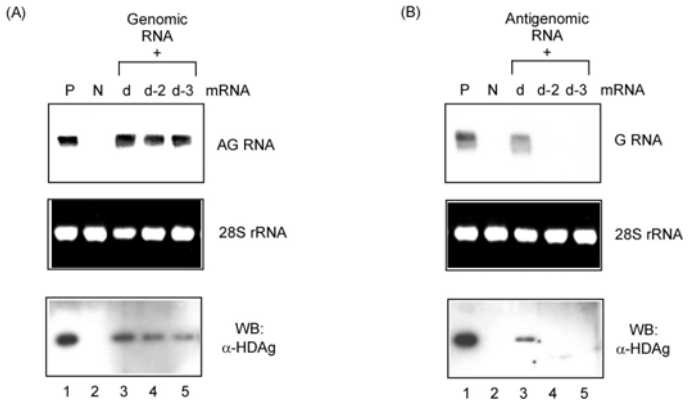
計 畫 主 持 人 ：陳培哲

報 告 附 件 ：出席國際會議研究心得報告及發表論文

處 理 方 式 ：本計畫可公開查詢

中 華 民 國 96 年 07 月 26 日

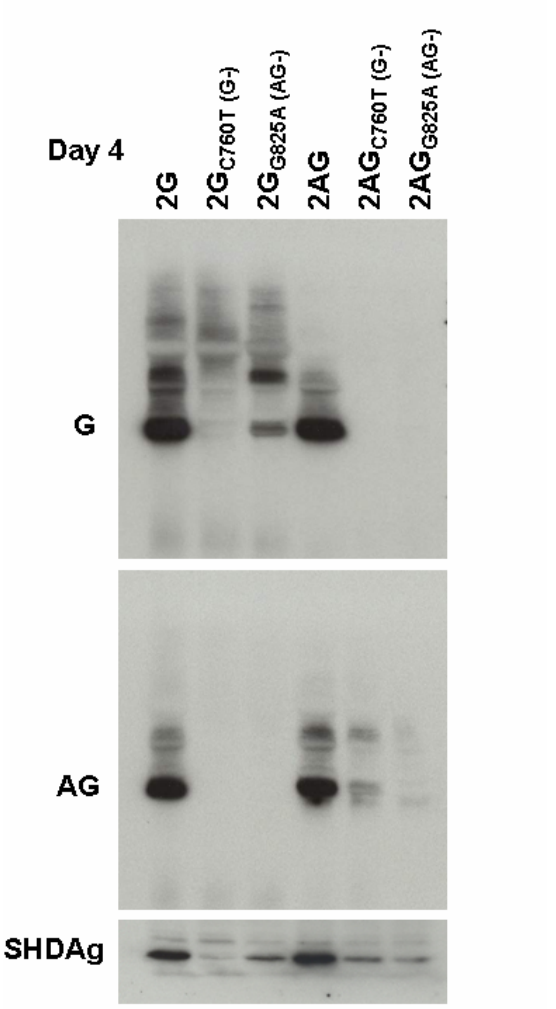
Hepatitis delta virus (HDV) is a small negative-strand RNA virus that contains one 1.7 kb single-stranded circular RNA. HDV particle also contains two isoforms of hepatitis delta antigen (HDAg). Small HDAg (SHDAg) is required for the replication of HDV, which is presumably carried by host RNA-dependent RNA polymerases. The exact molecular mechanism for HDV RNA replication is still obscure. A double rolling-circle mechanism for HDV replication has been proposed. Since HDV does not encode its RNA-dependent RNA polymerase and HDAg does not possess any RNA polymerase activity, the host RNA polymerase is likely adopted for HDV replication. Some recent studies suggested that replication of HDV RNA is carried out by two different cellular RNA polymerases including Pol II and polymerase I. The two RNA polymerases reside in different subcellular locations and HDV replication has to move accordingly. In fact, during the replication cycle, HDV RNP indeed shuttles between the nucleolus, nucleus, nucleoplasm and cytoplasm. To differentiate the intranuclear locations for HDAg on the viral RNA replication, we introduced a heterogeneous nucleolar localization signal (NoLS) from HIV rev protein to SHDAg to restrict its localization and then analyzed its effect on HDV replication. When SHDAg was fused with NoLS motif and almost localized in nucleoli, we found that the initiation of genomic RNA synthesis, but not that of antigenomic RNA synthesis, was abolished. Drug treatment by actinomycin D to release SHDAg-NoLS mutant from nucleoli could partially restore the replication of genomic RNA. These data suggest that subcellular localization of SHDAg affects its ability to support the initiation of HDV RNA replication and indicate the replication of two polarities of HDV viral RNA occur in different subcellular site.



Another part of our work is on the correlation of post-translational modification of SHDAg and HDV replication. Our previous study had demonstrated SHDAg could be phosphorylated at serine 177 and mutagenesis at this residue resulted in reduction of HDV genomic RNA synthesis. In the following experiments, we further characterized the function of the serine 177 phosphorylation of SHDAg in vivo and found the serine 177 is important for the interaction with RNA polymerase II. Mutation at serine 177 would lose the function to interact with the RNA pol II. Since pol II is suggested to be the polymerase responsible for HDV genomic RNA synthesis, the phosphorylation at serine 177 may play a critical role to modulate HDV RNA replication. ERK was identified as the kinase capable of catalyzing phosphorylation at serine 177. Following functional characterizations are undergoing.

Either HDV genomic or antigenomic RNA contain ribozyme activity. The crystal structure of HDV ribozyme had been identified, where C75 of HDV genomic ribozyme is critical for itself activity by *in vitro* assay. To address the role of HDV ribozyme in viral replication, we constructed the ribozyme-mutated HDV cDNA dimer driving by CMV promoter. Either wild type or mutant type of HDV cDNA

expressing plasmid was transfected into Huh7 cells, and total RNAs and total proteins were analyzed at post-transfection day 4. The single nucleotide mutation significantly affects the activity of HDV replication, no matter the mutation was in genomic or antigenomic sense. The results demonstrate that impairment of ribozyme activity in either HDV viral RNA abolishes HDV replication.



出席國際學術會議心得報告

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出國人員姓名 服務機關及職稱	陳培哲 國立臺灣大學醫學院臨床醫學研究所教授
會議時間地點	June 1~5 ,2007 ,Chicago
會議名稱	**ASCO 年度會議**

§ 在 Prostate cancer 之 ADT（Androgen Deprivation Therapy）

預防性使用可減少 Prostate cancer（Pca）發生率 30%

Pca. Primary therapy 之後，PSA rising（rapid vs. slow），ADT 並無太好的效果。

AR reactivation and Pca relapse（在 ADT 之後）的 mechanism：這時 AR（nuclear）表現很高，down-stream genes（to PSA）也升高，只是比 AR dependant stage 的幅度低。在細胞內，AR 以（AR-Hsp90-HDAC6）complex 存在。

AR mutations in relapse：

3/23（monotherapy）→ 9/21（combined therapy），可以加強與 antagonist 的結合而活化 AR。

pJer81→ stabilizy AR

Src→ phosphorylate tyrosine residue of AR

§ AR + Co-regulators

Co-activators 不見得 specific to AR（PR 也可以）

分爲 N-terminus
DNA binding
Ligand binding
} 互動 domains

現在對 co-regulators 的偵測及了解較多了，包括 SRC-1、TIF-2 等。

Anti-androgen withdraw syndrome：停用 anti-androgen 後，有些 Prca 會改進。

和 p300 有關

Gelsolin，Cdc25 等，都屬 co-factors

Other ARA54,55，etc.

§ Targeting Hsp90 to manipulate AR

Geldanamycin 可直接結合 Hsp90（N-term），抑制它的功能。

Hsp90 client protein：Src、HER2、Raf-1、AKt、AcK…等，都可以與 AR 互動。
（AR 可能需 co-chaperone FKBP52）

↓

爲現成的 inhibitor，但須改變其專一性

§ Clinical science symposium：

在用先進影像檢查 MRI，來檢查 breast cancer 的多發展性或 early detection，確比 echo 及 CT 好，但是之後能否接續合適的臨床治療，證實之後的 clinical outcomes 更好，仍待證明；有些是直覺上比較好，但後來的臨床研究卻證實並無臨床效應，醫師在這一方面尤其需注意。

June 3 , 2007. 下午

共有五個演講，首先是由 Susan G. Komen 乳癌基金會的主席（Susan 的姐姐）敘述癌症的社會影響，及癌症研究的成果及遠景，講到目前美國社會對癌症的忽視及 complacency，缺乏對癌症的危機及努力的熱情及資源投入。There is a great divide，需要改變美國人的 Culture：to discover and to deliver。

§ Plenary Session

(1) Sorefenib in Advanced HCC； by Dr. Lovett

HCC	Early	30%	Curative surgery
	Intermediate-stage	20%	TACE
	Advanced	40%	New therapies
	End-stage	10%	Supportive care

Sorafenib：raf kinase/MEK/MEK pathway inhibitions
Induce apotosis in HCC xenograft model
Block VEGF＆PDGR pathways

Randomized trial：
Stratified by ① macroscopic PVT
② ECoG Score 0-2
③ Geographic regions
共－Sorafenib 2 bid 每天 vs. Placebo

Histology HCC
Advanced HCC（at late or metatatic HCC）
ECOG Score 0-2
Child-Pugh A
No prioer systemic therapies

End-point：over-all survival（90% power to create 40% improvements）

另有 TTSP

病例：902 例 → screening 後 → 602，再 randomized－299（S）
303（P）

2006 Oct 17, 在 2nd interim analysis（321 deaths）
2007 Feb. DMC 建議 stopped trial

Regular 86% Europeans， 9% N. America
HCV 29% ， Alcohol 26% ， HBV 19% ， other
Child P A 95%
Surgery 20%
Local ablation therapies 40%
BSLC C：82%
ECOG 0-1：92%
Vascular / exterhepatic spread：70%（30% not）

§ **OS（ITT）：median**

10.7 months，placebo 7.9 months，HR：0.69
TTP：median，5.5 months，placebo 2.8 months

Response assessant RECIST

	S	P
PR	2.3%	0.9%
SD	71%	67%
PD	18%	24%
Progression-free rate at 4 months	62%	42%

TTSP：無差別
Subgroup：有些仍未確認到 statistical specification，只有 ertrahepatic 有無兩組分別。
Advance effects：tolerance 主要爲 diarrhea，及 Hand and foot disease。
{ 較大的問題是有多少是否能做 TACE？
{ 比 TACE 是否較好？
Conclusion：first systemic therapy for advanced HCC patients

§ **Discussant： P. Johnson**

第五位（incidence），3rd，death
62 萬人 59 萬人

HCC：需 systemic therapy－ ① adjuvant
② Advanced cares，systemic

Systemic therapy；但目前仍未有成效

Poxornbind（7.5→10.6 wks）

其他 cytotoxic，10~25%，response rate，沒有 improve survival

Sorafenib：

Phase Ⅱ OS 9.2 months，TTP 4.2 months

Phase Ⅲ ・Doxorubin ・D＋Sorafenib } 完成，正在分析中

SHARP trial：{ Child A 95’~95% / Europe 85% } 需注意 limitation

{ OS，TTP，PCR 有差別 / 可是 TTSP 無差別 }

Toxicity：差不多（54% vs. 52%）

建議：

(1)Local ablation or resections patients → Sorafenib 用爲 adjuvant therapy

(2)TACE 之後，±Sorafenib

(3) ±Sorafenib 之前再用 TACE

{
- 對症狀有無用處？
- 另有 Child A，歐洲 cases
- 無 pharmacogenetic markers
}

{ 44% OS improvement：Dramaic / 2.7 months prolongation：modest / Solid area well-controlled stage }

Dawn of HCC Tx era → 一個開始及起步

§ 美國 Breast Cancer 下降的原因

在 2003 年美國女性的 breast cancer 發生率下降 7%，原因何在？

經過比對 2000 年及 2005 年的全國健康行爲調查表，發現美國女性使用 Hormone replacement therapy 由 26%降到 12%，而使用 mammography 來做乳癌篩檢的爲 83%vs.85%沒有太大增加，顯示 HRT 使用率減少，是美國女性乳癌快速下降的主要原因。因此可見早期直覺的想法，認爲 HRT 對於女性荷爾蒙減少的效益，太單純化，HRT 後有許多不利的效果，經過宣導，少用 HRT 之後，連乳癌發生率也隨之降低了，好的研究有時只是清除錯誤觀念，不當的藥物使用，而達到促進社會大眾健康的目的。